Preliminary Study of Low Gamete Viability in Adult Chum Salmon *(Oncorhynchus keta)* Held in Sea Pens at Deserted Creek, Hisnit Inlet, B.C.

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PRELIMINARY STUDY OF LOW GAMETE VIABILITY IN ADULT CHUM SALMON (*Oncorhynchus keta*) HELD IN SEA PENS AT DESERTED CREEK, HISNIT INLET, B.C.

by

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ABSTRACT

Lam, C. N. H., J. O. T. Jensen, and D. F. Alderdice. 1982. Preliminary study of low gamete viability in adult chum salmon (Oncorhynchus keta) held in sea pens at Deserted Creek, Hisnit Inlet, B.C. Can. Tech. Rep. Fish. Aquat. Sci. No. 1133: vii + 49 p.

Maturing chum salmon, intercepted on their approach to Deserted Creek, Hisnit Inlet, B.C., were held until mature in sea pens in the creek estuary (salinity: surface, 28.3-30.0‰; 1-3 m, 29.3-30.6‰) and in a shore pen (pool pen) in the creek mouth (salinity: surface, 15.4‰; 1-3 m 24.3-27.5‰) during the initial part of the spawning period (Phase I) and in the pool pen (salinity: surface, 0-0.5‰; 1-3 m, 0-0.75‰) during the final part of the spawning period (Phase II). In general, fertilization success was lower and more variable among eggs collected during Phase I, rising to an average of 97.4% during Phase II. The potential influence was examined of (1) the holding environment on adult maturation and gamete viability, and (2) the procedures involved in artificial spawning, short-term storage and transport of gametes, and their effects on subsequent survival of fertilized eggs.

Among the gamete collection procedures monitored, three significant indicators of dysfunction were recognized associated with holding maturing adults in sea water, all leading to lower and variable survival of collected gametes and fertilized eggs (Phase I). They include prespawning mortality in captive fish; presence of turgid, apparently water-hardened eggs in the coelomic cavity of ovulated females; and increased incidence of embryonic abnormalities in fertilized eggs. During Phase I, gametes from fish exposed to higher salinities also were exposed to higher temperatures between collection of gametes and egg fertilization. During that period, lower fertilization success was associated with higher temperatures in the stored eggs at fertilization (range, 9.2-14.3°C), with containers holding larger quantities of eggs (4.21-5.88 kg), and with higher incidence of turgid eggs among those collected (0.0-13.8%). The following had no measureable influence on fertilization success during Phases I or II: storage time of gametes in the body cavity of the adult (1-57 min); temperature of eggs at collection (8.8-16.0°C); storage time of eggs from collection to fertilization (2.9-7.9 hr); temperature of milt at fertilization (7.8-10.1°C); contact time of gametes from fertilization to activation (0.5-4.5 min); and the interval between activation of eggs and their transfer to incubators (0.5-4.0 min).

A number of experiments were conducted concurrent with the monitoring of collection procedures. Although salinities were high both in the sea pen and the pool pen during Phase I, mortality among fertilized eggs and the incidence of embryonic abnormalities in that period were significantly higher in crosses involving eggs--but not milt--from adults held in the sea pens where holding salinities were highest. Gamete storage temperatures (2-3, 10-11°C) and storage times (3 to 11-12 hr) had no significant effect on fertilization success (Phases I, II), similar to results obtained during the monitoring of gamete collection procedures. However, trends suggested that storage times greater than 12 hr at higher temperatures could result in

reduced gamete viability, a suggestion strongly supported in a companion study (reported elsewhere). Milt plasma and ovarian fluid characteristics (pH, osmolality, Na^+, K^+, Ca^{2+}, Mg^{2+}) were found to differ significantly between males and females held in the pool pen during Phase II, and variations within these parameters were not correlated with differences in fertilization success. A shortage of seapen adults prevented a similar comparison of seapen- and poolpen-held adults during Phase II. Finally, the amount of water used to activate eggs at fertilization (water-to-egg volume ratios of 0.25, 0.5, 1.0) had no significant effect on fertilization rates. Incidental observations (Phase II) indicated there was no relation between volume of ovarian fluid bathing coelomic eggs (fluid-to-egg ratios of 0:1 to 1.5:1) and subsequent fertilization success even though eggs at the lower fluid-to-egg volume ratio appeared "dry".

The data obtained, and the results of recent and concurrent studies elsewhere, lead to a hypothesis regarding the holding of mature chum salmon in sea water. It appears that the final stages of sexual maturation are concurrent with loss of ability to osmoregulate in sea water. Holding maturing chum salmon in sea water leads to prespawning mortality, presumably through osmoregulatory failure. Increased blood plasma and ovarian fluid osmolality and Na^+ concentration have been noted elsewhere under similar holding conditions, and the fertility of females so held was inversely correlated with osmolality and Na^+ and K^+ concentrations in the ovarian fluid. Hence a higher osmotic environment for coelomic eggs is associated with their reduced fertilizability. We concur: in the present study reciprocal crosses of seapen and poolpen females and males showed the main problem to reside in the seawater-held female. In addition, in this and in other studies, the presence of a lens of less saline water in the holding pens was associated with reduced prespawning mortality and higher egg viability.

A further suggestion arises from the results and concurrent studies elsewhere regarding viability of stored gametes. Provision for improved gamete respiration should increase the viability of gametes stored and transported over a longer interval. As a preliminary guide we recommend storage and transport (at the lowest practical temperature) of unfertilized eggs under an equal volume of air (1:1, v/v). For milt, the fluid-to-air ratio should be at least 1:4 based on this study, and preferably 1:10 (v/v), while maximizing the fluid-to-air interface area. The latter may be achieved by storing partly filled plastic bags of milt on their sides.

Key words: chum salmon, seawater maturation, gamete viability, osmoregulation.

RESUME

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Des saumons kétas en maturation, capturés au moment où ils se dirigeaient vers le ruisseau Deserted dans l'inlet Hisnit (C.-B.), ont été gardés jusqu'à maturité dans des enclos de mer dans l'estuaire du ruisseau (salinité: surface, 28,3 à 30,0 °/00; 1 à 3 m, 29,3 à 30,6 °/00) et dans un enclos de rivage à l'embouchure du ruisseau (salinité: surface, 15,4 °/00; 1 à 3 m, 24,3 à 27,5 °/00), pendant la partie initiale de la période de fraie (phase I), et dans l'enclos de rivage, pendant la phase finale de la période de fraie (phase II). En général, le succès de la fertilisation a été moins élevé et plus variable chez les oeufs recueillis au cours de la phase I, mais a atteint 97,4% en moyenne au cours de la phase II. On a étudié l'influence potentielle (1) de l'environnement sur la maturation des adultes et la viabilité des gamètes, et (2) des méthodes de fraie artificielle, d'entreposage à court terme et de transport des gamètes et de leur incidence sur la survie ultérieure des oeufs fertilisés.

Parmi les méthodes de cueillette des gamètes qui ont fait l'objet d'une étude, on a déterminé que trois indicateurs importants de dysfonction étaient associés à la stabulation marine d'adultes en maturation et tous trois entraînent une survie variable et inférieure des gamètes recueillies et des oeufs fertilisés (phase I). Il s'agit de la mortalité pré-fraie des poissons en captivité, de la présence d'oeufs turgides, apparemment durcis par l'eau dans la cavité coelomique des femelles pleines, et de l'incidence accrue de difformités embryonnaires dans les oeufs fertilisés. Au cours de la phase I, les gamètes des poissons exposés aux salinités élevées ont aussi été exposées à des températures plus élevées pendant la période entre la cueillette des gamètes et la fertilisation des oeufs. A ce moment, le succès moins élevé de la fertilisation a été attribué aux températures plus élevées des oeufs entreposés au moment de la fertilisation (variation, 9,2 à 14,3°C), au fait que les récipients contenaient de plus grandes quantités d'oeufs (4,21 à 5,88 kg) et à une plus forte incidence d'oeufs turgides parmi ceux recueillis (0,0 à 13,8%). Les facteurs suivants n'ont eu aucune incidence quantifiable sur le succès de la fertilisation au cours des phases I et II: période d'entreposage des gamètes dans la cavité corporelle de l'adulte (1 à 57 min), température des oeufs au moment de la cueillette, (8,8 à 16,0°C), période d'entreposage des oeufs entre la cueillette et la fertilisation (2,9 à 7,9 h), température de la laitance au moment de la fertilisation (7,8 à 10,1°C), période de contact des gamètes entre la fertilisation et l'activation (0,5 à 4,5 in) et intervalle entre l'activation des oeufs et leur transfert aux incubateurs (0,5 à 4,0 min).

Un certain nombre d'expériences ont eu lieu en même temps que la surveillance des méthodes de cueillette. Quoique la salinité ait été élevée dans les enclos de mer et l'enclos de rivage pendant la phase I, la mortalité des oeufs fertilisés et l'incidence de déformités embryonnaires au cours de cette phase ont été de beaucoup supérieures dans les croisements d'oeufs, mais non de la laitance, chez les adultes gardés dans les enclos de mer, où la salinité était la plus élevée. Les températures d'entreposage des gamètres (2 ou 3 à 10 ou 11°C) et les périodes d'entreposage (de 3 à 11 ou 12 h) n'ont eu aucun effet important sur le succès de la fertilisation (phases I et II), résultats semblables à ceux obtenus au cours de l'étude des méthodes de cueillette des gamètes. Toutefois, les tendances portent à croire qu'un entreposage de plus de 12 h à des températures supérieures pourrait réduire la viabilité des gamètes, hypothèse fortement appuyée par une étude complémentaire (exposée ailleurs). Les caractéristiques de plasma de la laitance et du fluide ovarien (pH, osmolarité, Na^+, K^+, Ca^{+2}, Mg^{+2}) diffèrent de façon significative entre les mâles et les femelles gardés dans les enclos de rivage pendant la phase II, et les variations de ces paramètres ne sont pas en corrélation avec des différences dans le succès de la fertilisation. L'insuffisance du nombre d'adultes gardés en enclos de mer ne permet pas une comparaison semblable des adultes en phase II gardés dans les deux types d'enclos durant la phase II. Finalement, le volume d'eau utilisé, pour activer les oeufs au moment de la fertilisation (quotients du volume d'eau par rapport au volume d'oeufs: 0,25, 0,5, 1,0) n'a aucune incidence significative sur les taux de fertilisation. Des observations fortuites (phase II) révèlent qu'il n'y a aucune relation entre le volume du fluide ovarien baignant les oeufs dans la cavité coelomique (quotients du volume de fluide par rapport au volume d'oeufs: 0:1 à 1,5:1) et le succès ultérieur de la fertilisation, même si les oeufs où le quotient du volume de fluide par rapport au volume d'oeufs est le moins élevé semblent "secs".

Les données obtenues et les résultats d'études récentes et concomitantes réalisées ailleurs mènent à une hypothèse relative à la stabulation des saumons kétas adultes dans l'eau de mer. Il semble que les stades finaux de la maturation sexuelle se produisent en même temps qu'une perte de la capacité de régulation osmotique dans l'eau de mer. La stabulation de saumons kétas adultes en maturation dans l'eau de mer entraîne la mortalité avant la fraie, probablement à cause d'une mauvaise régulation osmotique. L'augmentation de l'osmolarité et de la concentration de Na^+ dans le plasma sanguin et le fluide ovarien a été notée ailleurs dans des conditions semblables de stabulation, et la fertilité des femelles ainsi gardées était en corrélation inverse avec l'osmolarité et les concentrations de Na^+ et de K^+ dans le fluide ovarien. On associe donc un environnement où la tension osmotique est plus élevée pour les oeufs relâchés dans le coelome avec leur capacité réduite de fertilisation. Nous sommes d'accord; dans la présente étude, des croisements réciproques entre des femelles et des mâles en enclos de mer et en enclos de rivage ont prouvé que le problème principal se recontre chez les femelles gardées dans l'eau de mer. De plus, la présence d'une nappe d'eau moins salée dans les enclos est associée avec une mortalité réduite avant la fraie et une viabilité accrue des oeufs.

Une autre hypothèse ressort de ces résultats et d'autres études parallèles au sujet de la viabilité des gamètes entreposées. Des conditions permettant une meilleure respiration des gamètes devraient accroître leur viabilité au cours d'un entreposage et d'une transport prolongés. Nos recommandations préliminaires sont les suivantes: entreposer et transporter (à la plus basse température convenable) les oeufs non fertilisés en présence d'un volume égal d'air (1:1, v/v); pour la laitance, le rapport entre le volume d'air et de fluide doit être au moins 1:4, d'après les résultats de cette étude, et préférablement 1:10 (v/v) tout en maximisant la superficie de l'interface entre le fluide et l'air. On peut obtenir ceci en entreposant sur le côté les sacs de plastique en partie remplis de laitance.

Mots-clés: saumon kéta, maturation en eau de mer, viabilité des gamètes, régulation osmotique.

INTRODUCTION

As part of its hatchery operations, the Department of Fisheries and Oceans since 1979 has collected chum salmon (Oncorhynchus keta) gametes at Deserted Creek, Hisnit Inlet, Vancouver Island, B.C. for transfer to the nearby Conuma River hatchery (Fig. 1). Generally, these gametes have exhibited low viability and highly variable fertilization success. Deserted Creek normally maintains a large, viable spawning population (Glova and McCart, 1979), suggesting that the cause of poor hatchery survival of fertilized eggs probably is not related to the quality of the wild spawning stock. Hence, the cause of the low egg viability likely is associated with manipulative procedures -- with some aspect of the adult holding environment prior to spawning or with some of the operations involved in collection of the gametes and their transportation prior to egg fertilization.

To ensure a supply of gametes, the hatchery each year has captured adult chum salmon by seining in Hisnit Inlet early in the approach period prior to the natural spawning period (Fig. 1). The captured fish are transferred to and held in seawater pens (sea pens) in the estuary near the mouth of Deserted Creek (Fig. 2) until they are ready to spawn. Adults also have been captured later in the spawning period directly from a deep pool in the mouth of Deserted Creek; there they may be spawned directly or held in a pen located in the pool (pool pen).

The technique of allowing captured adults to mature in sea water also has been employed in Alaska, Washington, and Oregon, often resulting in low and highly variable egg survival (Nosho, 1981). The factors associated with this low and variable egg survival currently are not well understood. It is suspected from Nosho (1981), Sower and Schreck (1982) and from examination of Conuma River hatchery records that they include osmotic stress in the adults during maturation in sea water, stress due to confinement and handling, disease, high water temperature, and conditions of gamete storage prior to and during their transfer from the capture site to the hatchery.

In the fall of 1981 a preliminary investigation was undertaken to determine the factors associated with the low viability of the gametes from the Deserted Creek stock. Accordingly, adult holding conditions were monitored, as well as the spawning operation--including the collection of gametes, their transfer to the hatchery and subsequent fertilization. In addition, a series of concurrent on-site experiments was carried out to examine the effects of seawater holding of adults, gamete storage conditions, and as a measure of the condition of the adults, the ionic composition of ovarian fluid and milt in relation to fertilization success of the gametes.

MATERIALS AND METHODS

LOCATION OF NET PENS AND SOURCE OF GAMETES

Deserted Creek drains Deserted Lake into the head of Hisnit Inlet (Fig. 1). It is 0.8 km long, and its total length is under tidal influence during periods of spring tides. All five species of Pacific salmon spawn in the creek, chum salmon being the most abundant. Visual and mark-recapture estimates of the chum spawning population range from 35,000 to 74,000, far in excess of the capacity of the available spawning area in the creek (Glova and McCart, 1979).

In 1981, gametes were collected from two sources. One group of spawners was captured by a chartered commercial seine boat, from October 12 to 16, at the head of Hisnit Inlet. These fish were carefully transferred to three 3 x 3 x 3-m floating net pens anchored in Deserted Creek estuary at a depth of 12 m (Fig. 2). Adult density in these sea pens was 450 fish per pen at a sex ratio of approximately 1:1. All other adults were seined from the deep pool in the mouth of Deserted Creek and held in another 1.2 x 1.2 x 2.4-m pen located in the same pool (Fig. 2) at a depth of 2-3 m. Fish density and duration of holding prior to spawning were not strictly controlled at the latter site. Fish held at the seapen site were spawned on October 24 and 26; those from the pool site were spawned from October 24 to November 4. A summary of the dates or duration of the monitoring procedures and experimental trials conducted is provided in Table 1.

MONITORING OF HOLDING CONDITIONS AND SPAWNING OPERATIONS.

Salinity, temperature and dissolved oxygen were measured at the surface and at 1-m intervals to 3 m below the surface at the sea pen and pool sites, using a YSI salinity meter (model 33) and a YSI dissolved oxygen meter (model 51B). Air temperature was measured with a mercury thermometer. Precipitation was recorded daily at the Conuma River hatchery, a distance of 7.5 km (by air) from Deserted Creek. Estimates of adult prespawning mortality in the sea pens were made on October 24 and 26.

The general spawning procedure, conducted by the hatchery staff, consisted first of dispatching the spawners by a blow on the head followed by cutting of the gill arches to bleed the carcasses prior to gamete collection. Eggs were collected in 22.5-L buckets lined with black plastic bags (eggs of 5 females to one bucket). As they were filled, the buckets were sealed and floated in the estuary or Deserted Creek until they were transported to the incubation facilities at the hatchery. Milt was expressed into quick-sealing plastic bags (Whirl-pak®) of various sizes ranging from 8 to 24 oz (~ 225 to 700 ml) (milt of one male to one or more bags), with an air-to-milt ratio in the sealed bags ranging from 1:3 to 4:1. The bags of milt were stored in coolers with re-usable artificial ice (Frig-pak®). The latter represented a

change in procedure from previous years when milt samples also were placed in sealed buckets handled in the same manner as the unfertilized eggs. The gametes then were transported by boat and truck to the hatchery. During transport, the buckets of eggs were exposed to air temperatures. Upon arrival, the containers were placed in an 8°C water bath until fertilization was carried out.

Three 10-15-ml aliquots of milt (3 bags) were used to fertilized each bucket of eggs (approximately 12,000 eggs per bucket). The mixture was hand-stirred gently and left undisturbed for about one minute. The fertilized eggs then were poured into 3 L of water in another bucket for activation. The activated eggs were placed carefully into incubation units, either modified Atkin's boxes or free-style boxes.

To facilitate monitoring during these operations, the majority of the gametes were placed in labelled containers. Information on individual egg and milt samples was obtained, including (i) length of time gametes were held in the body cavity of dispatched adults prior to collection, (ii) duration of gamete storage from collection to fertilization, (iii) duration of gamete contact at fertilization, (iv) duration of the period between activation and transfer to incubation boxes, (v) temperature of gametes at the time of collection and at fertilization, and (vi) weight of eggs (kg) in each bucket for enumeration estimates. In addition, the proportions of eggs of unusual appearance were estimated from 40-ml subsamples before activation. In particular, these included turgid eggs that appeared to be "water-hardened" -- those swollen and having a hardened zona radiata and a pale yellow yolk with coalesced oil globules.

To estimate egg viability, a 40-ml egg sample was taken after each bucket of eggs was fertilized. These egg samples were incubated, separately, to blastopore closure. At that time, the eggs were preserved in Stockard's solution for later examination to determine percentage survival, indicative of gamete viability at the time of fertilization. Incidence of abnormal embryonic development also was recorded.

ON-SITE EXPERIMENTS

General fertilization and response evaluation procedures.

Unless stated otherwise, the following procedures were used in all experiments. Replicated (3) 40-ml egg samples (~ 150 eggs/replicate) were fertilized in 150-ml glass beakers using 0.5 ml of milt per replicate. After water activation, using about 100 ml of water per replicate, eggs were placed in 5 x 5-cm compartments in incubation trays (Heath Techna Corp.®) and allowed to develop to easily recognizable embryonic stages, ranging from early cell division to the advanced eyed stage (stage 23, Vernier 1969). All eggs then were placed in Stockard's solution for later examination of embryonic viability and incidence of abnormalities.

Embryonic abnormalities were categorized into three groups: deformed--(i) absence of head or tail, hypotrophy or hypertrophy of embryonic

tissue, unorganized development with lobes of tissue observed in affected regions, irregular spinal curvature; (ii) twins--identical twins of equal size and development with both embryos sharing the same yolk, abnormal twins with deformed body parts or one common head or tail; and (iii) small embryos 1/2 to 3/4 the length of normal embryos at the same stage of development.

To evaluate differences between treatments, the arcsin transformation was applied to proportions of normal and abnormal embryos in each egg sample. Mean responses and their standard deviations (SD) or confidence intervals (CI) then were compared. Least squares regression analysis also was conducted to test for possible correlations between certain conditions or procedures and subsequent fertilization success or abnormal embryonic development. These procedures, where appropriate, also were applied to information obtained from the monitoring of hatchery operations.

Comparison of viability of gametes collected at the seapen and pool sites.

This experiment was conducted on October 25, 1981. Salinity, temperature, and dissolved oxygen measurements of a 3-m water column were recorded at both sites. Gametes from five females and five males were pooled according to sex and source. Upon arrival at the hatchery, four crosses were made, with seapen (S) and poolpen (P) gametes--S ♀♀ × S ♂♂, S ♀♀ × P ♂♂, P ♀♀ × S ♂♂, and P ♀♀ × P ♂♂. Fertilization success was evaluated by preserving the eggs at the advanced eyed stage.

The effect of chilling and ambient temperature on short-term storage of gametes.

To determine the effects of gamete storage temperature and duration of storage, four crosses were conducted involving ambient temperature (A) and chilling with ice (C)--A ♀♀ × A ♂♂, A ♀♀ × C ♂♂, C ♀♀ × A ♂♂, and C ♀♀ × C ♂♂. Each of these crosses was made at up to five storage intervals ranging from 3 to 12 hr. Pooled eggs from seven females and pooled milt from six males were divided into two groups, for (i) storage at ambient temperature and (ii) chilling with ice. Temperature of the pooled gametes was measured at the time of collection and at the time of fertilization.

Two tests of the influence of storage temperature and duration were conducted. The first test, on October 23, used gametes collected from adults held in the sea pens; the second, on November 2, used gametes collected from adults captured in the pool. In addition, on November 2, a further test was conducted. The object of this additional test was to provide an untransported control (at ambient temperature) for comparison of fertilization success with that for the gametes from the pool site transported to the hatchery on November 2. For the additional test, approximately 400 ml of eggs were fertilized about 30 min after collection using 10 ml of milt. These eggs were divided into two subsamples, which were placed in baskets and incubated in Deserted Creek. The subsamples were preserved after 20 hr of incubation (8-cell stage).

Ionic composition of ovarian fluid and milt plasma.

On November 4, gametes collected from seven females and six males, captured in the pool, were stored separately and transported to the Conuma River hatchery. Subsamples of ovarian fluid and milt were taken for visual inspection and chemical analysis before the full series of crosses (7♀♀ x 6 ♂♂ = 42 crosses) was conducted. pH measurements were obtained with an Orion specific ion meter (model 401). Milt was centrifuged at 11,000 RPM for 2 min in a centrifuge (IEC model MB) to obtain milt plasma samples. Duplicate 1-ml samples of ovarian fluid and milt plasma were stored in clear polystyrene sample cups at or below 5°C. Analysis for Na^+ and K^+ was performed using a Turner flame photometer (model 510), while Ca^{2+} and Mg^{2+} concentrations were measured with a Jarrell Ash atomic absorption flame emission spectrophotometer (model 82-516). Osmolality was measured using a Wescor vapor pressure osmometer (model 5100C). Egg samples from the 42 crosses were preserved at stage 18 (caudal bud free) (Vernier 1969). One of the seven females was partially spawned and did not provide sufficient eggs for full replication.

An additional test was conducted on November 3 to determine the effect of altering the volume of water used for egg activation. Three treatments consisting of water volume to egg volume ratios of 0.25, 0.5, and 1.0, were conducted on samples of eggs from three females, fertilized with pooled milt (0.5 ml per 40 ml of eggs) from three males, all from fish captured in the pool. Duration of fertilization (from insemination of eggs to addition of water) and activation (addition of water to transfer to incubators) was standardized at 0.5 min and 2.0 min, respectively. Eggs were preserved at stage 18 for later examination.

RESULTS

HOLDING CONDITIONS AND SPAWNING OPERATION

Salinity.

From October 23 to 25, salinity in the sea pens was high (Table 2), ranging from 28.3 at the surface to 29.3-30.6‰ in the 3-m water column. At the pool site, the water was noticeably stratified and highly saline; salinity measurements ranged from 15.4‰ at the surface to 24.3-27.5‰ in the 3-m water column. Salinity measurements were not made on October 26 and 27, due to equipment failure. During these latter two days, significant precipitation was recorded (Table 3). This resulted in significant changes in the salinity profile at both the seapen and the poolpen sites when measurements were resumed on October 28. The pool site had now become substantially freshened, with a salinity of 0.5‰ throughout the 3-m water column on October 28 and 0.0‰ from October 29 to the end of the spawning period. Salinity at the seapen site (October 29) became stratified, salinity being 11‰ at the surface and 25.0 to 29.0‰ in the 3-m water column. This stratification continued until a 1-m freshwater surface lens was recorded on November 2 and 3.

Temperature.

The two adult holding sites showed minor differences in water temperature (Table 4), the seapen site generally being about 1.6°C warmer than the pool. This probably was a result of greater water movement and abundant shade at the pool site, the latter factor contributing to the lower air temperature as well. A reduction in water temperature at both sites also occurred in response to the rainfall commencing on October 26. Stratification was apparent at both sites, temperature increasing with water depth.

Dissolved oxygen.

Dissolved oxygen levels at the two holding sites were inversely related to salinity and temperature (Table 5). The seapen site averaged ($\bar{x}$ ± 1 SD) 8.6 ± 0.1 ppm dissolved oxygen while the pool averaged 10.6 ± 1.4 ppm. Stratification in the water column also was observed, particularly on October 26 in the pool, where dissolved oxygen ranged from 11.1 ppm at the surface to 7.4 ppm at a depth of 3 m. Tidal influence in the pool, recorded on October 28 and 29, resulted in lower dissolved oxygen levels at high tide than at low tide on a given day. Differences ranged from 0.3 ppm to 0.9 ppm at the same depth.

Prespawning mortality.

High prespawning mortality was observed in all three sea pens at the time of gamete collection (October 23-26). Adult mortality was approximately 40% in two of the pens and about 20% in the third. Monitoring of holding conditions did not commence until October 23. Hence, the holding environment is not known from the date of capture (October 12-16) until October 22. It is assumed, however, that salinities would have been in the range of those recorded for October 23-25 (28.5 - 30.6 ‰ S).

Spawning operation.

Tabulated information for eggs and milt collected by the hatchery staff, including air and water temperatures at time of gamete collection, time delays, and temperatures of gametes at the time of collection and arrival at the hatchery, is listed in Appendix Tables I and II. Estimates of fertilization success for individual buckets of eggs, fertilized with milt pooled from several males, are listed in Appendix Table III. The table also includes the temperature of gametes at fertilization, percentage of turgid eggs, time intervals for the fertilization procedure, and the embryonic stage at which evaluation of fertilization success was conducted.

From estimates of fertilization success, it appears that two phases of egg viability (Phases I, II) were exhibited during the egg collection period (Fig. 3). Phase I showed a lower mean viability for gametes from the sea pens, and a trend of increasing viability with time at both sites. High variability in gamete viability also was observed, which tended to decrease with time. The transition to Phase II occurred near October 27. Phase II was characterized by high gamete viability (mean fertilization success greater

than 97%) and low variability. In addition, only fish from the pool site, in which the water now essentially was fresh, were used during Phase II.

Among the parameters listed in Appendix Table III, significant correlations ($P < 0.05$) were obtained in Phase I between fertilization success and (1) percentage of turgid eggs before fertilization ($r = -0.504$), (2) temperature of eggs at fertilization ($r = -0.513$), and (3) weight of eggs in a bucket ($r = -0.489$) (Table 6). Occurrence of turgid eggs was more frequent and generally of greater magnitude during Phase I of the spawning period, and among the adults held in sea pens (Fig. 4). During Phase II, no significant correlations involving percent fertilization success were obtained with any of the parameters recorded (Table 6).

ON-SITE EXPERIMENTS

Viability of gametes from seapen and pool sites.

The adult holding conditions on October 25, the time that gametes were collected, indicated that the spawners at the seapen site were exposed to somewhat higher salinities and temperatures than at the pool site (Tables 2, 4). Surface salinity at the seapens (30.0‰) was 14.6‰ higher than at the pool site (15.4‰), while smaller differences, ranging from 3.1 to 6.2‰ were recorded at subsurface depths to 3 m at the two sites (Table 2). Similarly, surface temperature at the sea pens (13.6°C) was 2.4°C higher than at the pool site (11.2°C), with subsurface differences ranging from 0.6 to 1.5°C (Table 4) at both sites. Dissolved oxygen at the surface in the sea pens (8.6 ppm) was lower than in the pool pen (11.1 ppm); at 1-m to 3-m depths, the levels in the pool pen were equal to or slightly lower than those in the sea pens (Table 5).

Difficulty was experienced in hand-expressing milt, because of its low volume and high viscosity, from 3 of the 5 males obtained from the sea pens. However gamete fertilizability at the two sites (Table 7) was found not to differ significantly ($F = 1.34$; $F.95(3,8) = 4.07$) although there was a tendency for fertilization rates to be higher among eggs obtained from females from the pool site. In addition, seapen gametes exhibited greater variation in fertilizability than those collected from the pool site. Furthermore, a significantly greater proportion ($F = 12.07$; $F.995(3,8) = 9.60$) of abnormal embryos (about 6-8%) were observed in preserved egg samples originating from the seapen site (Table 7,8). It is apparent that the observed embryonic abnormalities are associated with eggs collected from the seapen site since milt originating from the seapen site did not appreciably alter the resultant proportions of abnormalities in eggs from the pool site.

Gamete storage conditions.

The first test, conducted on October 23 (Phase I) with gametes collected from the seapen site, resulted in low viability and high variability (Fig. 5) for all treatment combinations of chilled (approximately 2°C) and ambient (approximately 11°C) storage temperatures and for storage times of 3 to 11 hr. Significant embryonic abnormalities also were observed later

($\bar{x}$ = 5.2%) in eggs that had been transported either chilled or at ambient temperatures. This general, low level of egg viability was similar to that observed for gametes collected by the hatchery staff during the initial part of Phase I of the spawning period (Fig. 3). This low level probably masked any differences that may have occurred due to storage conditions. No significant differences or trends in decreasing viability were observed between chilled (2°C) and ambient (11°C) storage temperatures ($F = 2.60$; $F.95(4,16) = 3.01$) involving five storage times from 3 to 11 hr ($F = 0.59$; $F.95(4,16) = 3.01$) (Fig. 5).

The second test, conducted on November 2 (Phase II) with gametes collected from the pool site, resulted in high viability (range 90.2-100%) among gametes stored either chilled (2.3°C) or at ambient (10.5 °C) storage temperatures for periods of 3 to 12 hr (Fig. 6). Two-way ANOVA showed no significant differences among any of the treatment combinations: (1) for storage duration ($F = 3.48$; $F.95(2,6) = 5.14$), or (2) for storage temperature ($F = 2.30$; $F.95(2,6) = 4.76$). These results are similar to those of the first test of seapen gametes (October 23). However, a trend in decreasing viability of approximately 8% was observed for gametes stored for 12 hr at the higher ambient temperature.

The eggs incubated in Deserted Creek, providing a "transportation" control for comparison with the gametes transported to the Conuma River hatchery, indicated that no significant loss in viability was incurred through transportation. Both groups of eggs, those incubated at Deserted creek and those transported to the hatchery, resulted in 100% survival (Table 9).

Ionic composition of ovarian fluid and milt plasma.

Table 10 presents measurements of pH, osmolality, and ionic concentrations in samples of ovarian fluid and milt plasma obtained from adults held in the deep pool (Phase II). Analyses showed (Table 11) that levels of pH, osmolality and concentrations of Na^+ and Ca^{2+} were significantly greater ($P<0.05$) in ovarian fluid than in milt plasma. In contrast, K^+ concentration was significantly greater ($P<0.01$) in the milt plasma (Table 11). Concentration of Mg^{2+} did not differ significantly between the two fluids ($F = 0.33$; $F.95(1,11) = 4.84$).

Analysis (Table 12) shows that a significant correlation ($r = 0.979$, $P<0.01$) exists between fertilization success and Mg^{2+} concentration in the milt plasma. Further analyses showed this correlation was associated with milt from male 4, which, when omitted from the analysis, removed the correlation ($r = -0.451$). The milt sample from male 4 was unusually viscous. When centrifuged, it yielded a low volume of supernatant fluid and the fluid (milt plasma) was translucent. All other milt plasma samples were clear.

Finally, two-way ANOVA showed that there was no significant difference in fertilization success among the six males including male number 4 ($F = 1.12$; $F.95(5,30) = 2.53$), and the seven females ($F = 0.956$; $F.95(6,30) = 2.42$). Fertilization success for all crosses was high, averaging 98.3%, (Fig. 7).

The quantity of ovarian fluid, although not measured, was observed to be low (eggs appeared dry, the fluid-to-egg volume ratio being 0:1) for eggs from females 5 and 6, while female 7 had an exceptionally large ovarian fluid-to-egg volume ratio (about 1.5:1). However, the quantity of ovarian fluid in these females was not correlated with fertilization success (Fig. 7). Finally, altering the volume of water used to activate inseminated eggs had no significant effect on fertilization success ($F = 0.05$; $F.95(2,4) = 6.94$) (Fig. 8).

DISCUSSION

Salinity, temperature, and dissolved oxygen measurements at the netpen sites are indicative of those conditions that captured adult chum salmon would be exposed to during the final stages of maturation (Tables 2, 4, 5). These conditions were well within the levels of tolerance for adult chum salmon. Yet the high percentage of prespawning mortality occurring in the seapen adults indicates that a serious problem existed in the seapen environment. In addition, the higher incidence of embryonic abnormalities (Table 7, 8) in gametes collected from the seapen site (frequency of 6-8%) is an indication that the quality of gametes was affected adversely by continued exposure of the adults to sea water. However, the tests to compare the viability of gametes collected from the seapen and pool sites (Table 7) did not show significant differences in fertilization success, probably because both sites had rather similar salinity environments during Phase I of the spawning period. A shortage of seapen-held fish precluded the possibility of repeating this and related tests during Phase II of the spawning period.

We suspect, however, that exposure of maturing adults to sea water during holding influences osmoregulatory ability, leading to subsequent deterioration in gamete quality. It is assumed that adults captured before or during phase I of the spawning period would not be fully mature, and that crosses made in that period would tend to show lower and more variable rates of fertilization success in keeping with a substantial variation in degree of maturity of the gonads involved. If this were so, then Figure 3 would be indicative of the normal maturation process, with increasing rates of fertilization and decreasing variance with time.

A further difference between mean fertilization rates in Phase I (Fig. 3) is suggested by the apparent 2-day lag of seapen egg fertilization rates, compared with those from the pool pen, between October 24-26. This difference would suggest that the exposure to higher salinities in the seapen fish in some way may have inhibited and prolonged the process of attaining final stages of gonad maturation in the seapen fish. A number of pieces of evidence provide some insight into the problem. Sower and Schreck (1982) maintained maturing coho salmon (*O. kisutch*) in sea water (30-32 ‰) after their entry into a holding facility. Blood serum sodium and osmolality in both males and females in sea water retained approximately their initial levels for the following 16 days. Levels in a companion group in fresh water dropped substantially over the same period. Over the next 7 days significant

increases occurred in serum sodium and osmolality levels in the seawater-held fish; concurrently, mortality increased and remained high over that period. During the same period sodium and osmolality levels varied little in the companion group in fresh water. These data are of interest in the fact that the beginning of increased serum sodium and osmolality levels and increased mortality in sea water was related to the beginning of oocyte maturation, suggesting that maturation and osmoregulation in some way are antagonistic processes.

From Hirano et al. (1978) and Morisawa et al. (1979) it can be seen that blood serum, ovarian fluid and milt plasma levels of sodium, chloride and osmolality in O. keta follow similar decreasing trends following transfer of mature fish to fresh water. Approximately one-half of the total decrease in ovarian fluid and milt plasma ion levels over a 7-day period occurred within 12 hr after transfer. In addition, Sower (1980) (cited in Sower et al. 1982) found that egg sodium and osmolality levels were higher in females in sea water than those from fish in fresh water. Hence during oocyte maturation, salinity of the external medium influences sodium and osmolality levels in the blood, thence the ovarian fluid and the egg in the female, and the milt plasma in the male. These relations may be seen in the data compiled in Table 13 for O. keta. In addition Stoss and Fagerlund (1982) found for females held in sea water that the elevated osmolality, Na^+ and K^+ levels shown in the table were associated with a significant reduction in egg fertilization potential, compared with eggs from the similar group of females they held in fresh water. Therefore the holding of adult chum salmon in sea water during oocyte maturation is seen to result in elevated body fluid ion levels (particularly sodium and chloride), increased adult mortality and decreased egg viability at fertilization.

Sower and Schreck (1982) also reported the simultaneous occurrence of unovulated, ovulated and overripe coelomic cavity eggs in females held in sea water. Our observations on the proportions of turgid eggs (Fig. 4) occurring in fish held in the sea pens and pool pen during Phase I, when the deep pool also was saline (>15.4 ‰S, Table 2), would suggest the presence of a similar but smaller problem associated with adult osmoregulation and maturation in the poolpen fish. In a somewhat similar trial, Wertheimer and Martin (1981) captured maturing coho salmon (O. kisutch) in a river and penned them in an estuary where a fresh/brackish surface water lens up to 1 m in depth existed intermittently. In these fish there was low prespawning mortality (0.8%) and high gamete viability ($\bar{x}$=96%). Their results suggest that continuous exposure to fresh water is not necessary for final maturation of coho salmon gametes, and that maturation may proceed normally where some access to less saline water is available as in the presence of a freshwater lens. We suspect the lens of low salinity water present in the pool pen during Phase I may have averted a major osmoregulatory problem, a problem that would have been resolved by the rapid drop in salinity in the pool pen at the beginning of Phase II and the rapid internal adjustment of osmoconcentration occurring in the adult after transfer to fresh water (Hirano et al. 1978; Morisawa et al. 1979). This agrees with our findings that there was no correlation between egg viability and variations in ionic composition of ovarian fluid or milt plasma collected from poolpen fish during Phase II. Further, the milt plasma and ovarian fluid characteristics of these fish during Phase II compare favorably with those for freshwater-adapted adults (Table 13).

The tests to determine the effects of transportation procedures on gamete viability indicated that chum salmon gametes were relatively resistant to short-term storage. Storage at ambient temperature ($\bar{x}$ = 10.5°C) for 12 hr did not cause a significant decline in fertilization success ($F = 3.48$; $F.95(2,6) = 5.14$), although a trend in declining viability appeared likely after 12 hr of storage (Fig. 6). In addition, significant correlations were observed between fertilization success and (1) temperature of eggs at fertilization, and (2) the number (weight) of eggs stored in a bucket (Table 6). This relation between storage time, temperature at fertilization and quantity of eggs stored per bucket requires some further examination. Jensen and Alderdice (MSa) stored chum salmon gametes at a series of constant temperatures for periods up to 360 hr. Sealed plastic bags were used to store small volumes of milt (2.25 ml) under large volumes of air (about 75 ml), providing an air-to-milt interface of about 300 mm^2/ml of milt. Eggs were stored in sealed plastic containers, which were opened and resealed frequently. Under such conditions the gametes were stored at 15°C for 19.6 hr (95% confidence limits--12.8, 30.0 hr) before incurring a 10% loss in viability. In contrast, a compilation of gamete storage data for Oncorhynchus from the earlier literature indicated that a 10% loss of gamete viability would be expected after 2 hr of storage at 15°C. The only variable that could be related to this large difference (19.6 vs 2.0 hr) is that associated with gas exchange in the stored gametes. In this regard it has been shown that oxygenation or aeration of stored milt (J. Stoss, West Vancouver, pers. comm.) leads to a substantial increase in storage time of viable sperm. In similar experiments using oxygen or air exchange it was found that fertilization success in stored unfertilized eggs (Salmo gairdneri) was highest in the upper of multiple layers of eggs where the opportunity for gas exchange was greatest (Püschel, 1979).

Therefore, the relations between fertilization success of stored chum salmon gametes, temperature of eggs at fertilization, and quantity of eggs stored per bucket are interpreted as follows. Metabolic oxygen requirements of gametes can have a marked influence on their viability under storage, particularly at higher temperatures. Under field conditions, gas exchange may be enhanced by providing a large interface area and a large volume of air (or oxygen) over the stored gametes; an air-to-gamete ratio of at least 1:1 is suggested for eggs. We believe the ratio for air to milt should be at least 4:1, and preferably 10:1. It should be possible to provide a greater air to gamete interface area by laying partly filled containers of milt on their sides.

In summary, a number of operating procedures were examined in the collection of gametes from a wild stock of chum salmon. The influence of these procedures was related to prespawning mortality, gamete viability and subsequent egg survival as follows.

Procedure	Effect
Holding maturing adults in sea water (28.3-30.6 ‰S)	
-prespawning mortality	increased
-presence of turgid, water-hardened body cavity eggs	increased
-incidence of embryonic abnormalities in fertilized eggs	increased
-fertilizability of eggs	reduced
-fertilization potential of sperm	no effect
Gamete collection, storage, transport; egg fertilization, activation (influence on fertilization rate)	
-storage of gametes in body cavity of adult (1-57 min)	no effect
-temperature of eggs at collection (8.8-16.0°C)	no effect
-storage time, collection to fertilization (2.9-7.9 hr) and (3-12 hr)	no effect
-temperature of stored milt at fertilization (7.8-10.1°C)	no effect
-temperature of stored eggs at fertilization (9.2-14.3°C)	inverse correlation
-quantity of stored eggs per container (4.21-5.88 kg)	inverse correlation
-gamete contact time, fertilization to activation (0.5-4.5 min)	no effect
-interval, egg activation to transfer to incubators (0.5-4.0 min)	no effect
-ratio, water to eggs, activation (0.25:1 to 1:1)	no effect
-ratio, ovarian fluid to egg volume in body cavity (qualitative observations only) (0:1 to 1.5:1)	no effect

We suspect that the problems indicated could be partly rectified or overcome as follows.

HOLDING MATURING ADULTS IN SEA WATER

It appears that in the chum salmon there is a progressive loss of osmoregulatory ability that occurs in association with the development of final stages of sexual maturation. When the maturing adult is held in sea water, it appears that osmoregulatory failure occurs, influencing blood ion levels and ultimately ion levels in ovarian fluid and the milt plasma. Osmoconcentration in coelomic cavity eggs also appears to rise, reducing their ultimate viability at fertilization. Evidence indicates that the vitelline or plasma membrane of the coelomic cavity egg is impermeable to Na^+ (Rudy and Potts 1969). We assume that elevated ovarian fluid ion levels ultimately lead to failure of the vitelline membrane, which could lead to the increased incidence of abnormalities observed in coelomic cavity eggs from seawater-held females, as well as their lower and more variable survival following bulk fertilization.

Therefore we suggest that holding maturing chum salmon at lower salinities or in the presence of a freshwater or lower salinity lens should reduce prespawning mortality, reduce the occurrence of turgid, water-hardened coelomic cavity eggs and increase egg fertilization rates.

QUANTITY OF STORED EGGS PER CONTAINER AND EGG TEMPERATURE AT FERTILIZATION

Short-term storage of viable chum salmon gametes appears to be improved markedly when allowance is made for their respiratory requirements. Reducing the storage temperature results in an exponential increase in storage time of viable gametes (Jensen and Alderdice MSa). Oxygenation of stored milt results in a substantial increase in storage time of viable sperm (J. Stoss, West Vancouver, pers. comm.) and fertilization success is highest in the upper layers of unfertilized salmonid eggs stored in bulk (Püschel, 1979).

Therefore we suggest that collected gametes be cooled to the lowest practical temperature for short-term storage. Further, we suggest that an air space be provided over the gametes--at least 1:1 (v/v) for eggs and at least 4:1 based on this study, and preferably 1:10 (v/v) for milt. In addition, milt should be stored so as to provide the greatest possible air-to-milt interface area.

One further cautionary note: activated eggs quickly become highly sensitive to mechanical shock (Jensen and Alderdice, MSb). They should be inserted in incubators within 5 min of activation to minimize loss from shock.

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Table 1. Summary of dates or duration of monitoring procedures and experimental trials conducted.

Procedure or experimental trial	Date or duration
Salinity measurements	Oct 23 - Oct. 25
	Oct 28 - Nov. 4
Temperature measurements	Oct 23 - Nov. 4
Dissolved oxygen measurements	Oct 23 - Oct. 29
Gamete collection by hatchery staff	Oct 23 - Nov. 4
Comparison of gametes collected at seapen and pool sites	Oct 25
Effect of storage time and temperature on gametes	
(1) first trial with seapen adults	Oct 23
(2) second trial with poolpen adults	Nov 2
(3) influence of transportation on gamete viability	Nov 2
Ionic composition of ovarian fluid and milt plasma	Nov 4

Table 2. Salinity measurements at seapens and pool sites. Also included are differences (Δ) between sites on the same day.

	Date (Day-Month)							
	23-10	24-10	25-10	28-10	29-10	2-11	3-11	4-11
Seapen site								
Time of day (hr)	14:00	13:00	11:00	-	15:30	15:30	12:00	12:00
Surface salinity (‰)	28.5	28.3	30.0	-	11.0	0.0	0.3	1.4
Subsurface salinity (‰)								
1 m	29.5	29.3	30.5	-	26.5	0.1	0.4	7.8
2 m	29.8	30.2	30.6	-	25.0	10.2	6.8	13.2
3 m	30.3	29.8	30.6	-	29.0	15.0	12.1	21.1
Pool site								
Time of day (hr)	-	-	14:00	11:00	12:00	-	14:00	13:00
Surface salinity (‰)	-	-	15.4	0.5	0.0	-	0.0	0.0
Subsurface salinity (‰)								
1 m	-	-	24.3	0.5	0.0	-	0.0	0.0
2 m	-	-	27.0	0.75	0.0	-	-	0.0
3 m	-	-	27.5	0.45	0.05	-	-	0.1
Difference (Δ) (seapen - deep pool)								
Time of day (hr)	-	-	-3.0	-	3.30	-	-2.0	-1.0
Surface salinity (‰)	-	-	14.6	-	11.0	-	0.3	1.4
Subsurface salinity (‰)								
1 m	-	-	6.2	-	26.5	-	0.4	7.8
2 m	-	-	3.6	-	25.0	-	-	13.2
3 m	-	-	3.1	-	29.0	-	-	21.0

Table 3. Record of daily precipitation, Conuma River hatchery, 9-Oct to 4-Nov, 1981.

DATE	OCTOBER								NOVEMBER			
	9-24	25	26	27	28	29	30	31	1	2	3	4
Daily precipitation (cm)	0.0	drizzle	4.9	4.8	2.2	1.6	4.0	12.1	4.1	2.2	1.8	0.0

Table 4. Air and water temperatures at seapen and pool sites. Also included are differences (Δ) between sites on the same day.

	Date (Day-Month)								
	23-10	24-10	25-10	26-10	28-10	29-10	2-11	3-11	4-11
Seapen site									
Time of day (hr)	14:00	13:00	11:00	12:00	-	15:30	15:30	12:00	12:00
Air temp. (°C)	15.0	17.0	13.0	10.1	-	6.0	12.1	8.9	10.0
Surface temp. (°C)	13.1	13.7	13.6	13.0	-	11.0	10.3	9.7	9.4
Subsurface temp. (°C)									
at 1 m	13.4	13.4	14.0	13.5	-	12.0	10.1	9.6	10.3
at 2 m	13.6	13.8	13.6	14.0	-	13.0	10.6	10.0	10.8
at 3 m	13.9	13.8	14.5	14.0	-	13.0	11.0	10.6	11.4
Pool site									
Time of day (hr)	17:00	13:30	14:00	14:30	11:00	12:00	-	14:00	13:00
Air temp (°C)	10.4	20.0	11.3	9.7	9.5	6.0	-	9.6	10.0
Surface temp. (°C)	10.2	11.0	11.2	13.0	9.0	9.5	-	10.2	9.1
Subsurface temp. (°C)									
at 1 m	-	-	12.8	12.5	9.0	9.6	-	10.3	9.1
at 2 m	-	-	13.0	12.5	9.0	9.6	-	-	9.0
at 3 m	-	-	13.0	-	9.3	10.0	-	-	9.3
Difference (Δ) (seapen-deep pool)									
Time of day (hr)	-3.0	-0.5	-3.0	-2.5	-	3.5	-	-2.0	-1.0
Air temp. (°C)	4.6	-3.0	2.7	0.4	-	0.0	-	0.7	0.0
Surface temp. (°C)	2.9	2.7	2.4	0.0	-	1.5	-	-0.5	0.3
Subsurface temp. (°C)									
at 1 m	-	-	1.2	1.0	-	2.4	-	-0.7	1.2
at 2 m	-	-	0.6	1.5	-	3.4	-	-	1.8
at 3 m	-	-	1.5	-	-	3.0	-	-	2.1

Table 5. Dissolved oxygen levels at the seapen and pool sites from October 23 to 29.

Date	Seapens						Deep Pool Site					
	Time of day (hr)	Tide	Surface (ppm)	Subsurface (ppm) 1 m	2 m	3 m	Time of day (hr)	Tide	Surface (ppm)	Subsurface (ppm) 1 m	2 m	3 m
23-10	14:00	high	8.6	8.6	8.6	8.4	-	-	-	-	-	-
24-10	13:00	high	8.5	8.6	8.5	8.5	-	-	-	-	-	-
25-10	11:00	high	8.6	8.5	8.5	8.5	14:00	high	11.1	8.6	7.9	7.4
26-10	12:00	low	9.0	8.0	8.6	8.6	14:30	high	9.0	9.0	9.0	9.0
28-10	-	-	-	-	-	-	10:00	low	11.9	11.9	11.8	11.9
	-	-	-	-	-	-	12:00	high	11.0	11.5	10.7	10.8
29-10	-	-	-	-	-	-	10:00	low	11.7	11.7	11.7	-
	-	-	-	-	-	-	13:30	high	11.4	11.4	11.4	11.4

Table 6. Correlation coefficients between percentage of fertilization success and the listed parameters monitored during the normal hatchery gamete collection operation in Phase I and II (* statistically significant, $P<0.05$). (Egg sample no. 1 of October 24 was considered as an outlier, and was omitted.)

Parameters correlated with fertilization success (arcsin transformed %)	Range	Phase I (Oct 24-26)		Phase II (Oct 28-Nov 4)	
		D.F. (n-2)	r	D.F. (n-2)	r
Time eggs held in body cavity (min)	(1-57)	17	+0.154	23	-0.079
Temperature of eggs at collection (°C)	(8.8-16.0)	17	-0.455	23	-0.120
Weight of eggs in bucket (kg)	(2.81-5.88)	16	-0.489*	18	+0.073
Storage time, collection to fertilization (hr)	(2.87-7.85)	17	+0.299	23	-0.191
Percentage of turgid eggs before fertilization (arcsin transformed %)	(0-13.8)	17	-0.504*	23	-0.236
Temperature of eggs at fertilization (°C)	(8.1-14.3)	17	-0.513*	17	-0.180
Temperature of milt at fertilization (°C)	(7.8-10.1)	17	-0.446	12	+0.470
Contact time of gametes, fertilization to activation (min)	(0.5-4.5)	17	-0.242	23	+0.190
Interval between activation and transfer to incubator (min)	(0.5-4.0)	17	-0.262	23	+0.347

Table 7. Gamete fertilizability and abnormal embryos resulting from seapen (S) and pool (P) crosses. Abnormal embryos are shown as a percentage of fertilized and developing eggs (Phase I).

Fertilizations	Eggs	Number sampled	Percent fertilized (%)	Percent abnormal embryos (%)
S ♀♀ x S ♂♂	Rep 1	83	78.3	7.69
	Rep 2	83	85.5	7.04
	Rep 3	84	92.9	10.26
	Mean	83.3	86.14	8.28
S ♀♀ x P ♂♂	Rep 1	77	90.9	5.7
	Rep 2	100	91.0	4.4
	Rep 3	82	87.8	8.3
	Mean	86.3	89.9	6.03
P ♀♀ x S ♂♂	Rep 1	84	92.9	2.56
	Rep 2	93	89.3	0
	Rep 3	87	92.0	0
	Mean	88.0	91.46	0.29
P ♀♀ x P ♂♂	Rep 1	87	88.5	1.3
	Rep 2	89	94.4	0
	Rep 3	83	92.8	0
	Mean	86.3	92.07	0.15

Table 8. Types of abnormal embryos and their occurrence in the four crosses performed between seapen (S) and pool (P) parents (Phase I).

Type of abnormality	Cross			
	S ♀♀ × S ♂♂	S ♀♀ × P ♂♂	P ♀♀ × S ♂♂	P♀♀ × P♂♂
Deformed	15	8	1	1
Twins	2	5	0	0
Small embryo	1	1	1	0
Total	18	14	2	1
Total number of eggs examined	250	259	264	259

Table 9. Fertilization success of gametes transported to hatchery before fertilization and those fertilized and incubated in Deserted Creek.

Treatments		Number of eggs sampled	Percentage of fertilization success (%)
Transported to hatchery	Rep 1	148	100
	Rep 2	151	100
	Rep 3	139	100
Incubated in Deserted Creek	Rep 1	111	100
	Rep 2	106	100
	Rep 3	132	100

Table 10. Measurements of pH, osmolality, and concentrations of Na^+, K^+, Ca^{2+}, and Mg^{2+} in samples of ovarian fluid and milt plasma from adults held in fresh water (Phase II).

Observations Specimen Number		pH	Osmolality (mOsm) mean ± 1 S.D.	Concentrations of cations (mM/L) Na^+ mean ± 1 S.D.	K^+ mean ± 1 S.D.	Ca^{2+} mean ± 1 S.D.	Mg^{2+} mean ± 1 S.D.
Milt plasma	1	7.80	292.0 ± 1.79	128.0 ± 0.0	31.9 ± 0.07	0.95 ± 0.07	0.95 ± 0.07
	2	7.80	290.0 ± 3.10	131.0 ± 0.0	24.1 ± 0.0	0.98 ± 0.04	0.68 ± 0.04
	3	7.75	309.3 ± 2.56	135.0 ± 0.0	33.1 ± 0.07	0.78 ± 0.04	0.88 ± 0.04
	4	7.95	276.7 ± 2.16	128.5 ± 0.71	30.2 ± 0.28	0.63 ± 0.04	2.18 ± 0.18
	5	7.90	296.2 ± 1.60	128.0 ± 1.41	31.6 ± 0.42	1.23 ± 0.18	0.85 ± 0.07
	6	7.80	307.5 ± 1.05	136.0 ± 0.0	31.2 ± 0.14	1.10 ± 0.0	1.05 ± 0.0
overall mean ± 1 SD		7.83 ± 0.075	295.3 ± 12.1	131.1 ± 3.61	30.35 ± 3.2	0.94 ± 0.22	1.10 ± 0.54
Ovarian fluid	1	8.28	298.8 ± 1.47	163.0 ± 1.41	3.02 ± 0.07	3.80 ± 0.0	0.80 ± 0.14
	2	8.22	296.3 ± 2.07	160.5 ± 0.71	3.22 ± 0.06	2.80 ± 0.0	0.85 ± 0.14
	3	8.30	300.7 ± 1.03	161.5 ± 0.71	4.63 ± 0.93	3.90 ± 0.28	1.58 ± 0.11
	4	8.22	307.5 ± 1.87	166.5 ± 2.12	2.93 ± 0.06	3.40 ± 0.0	0.95 ± 0.07
	5	8.18	304.1 ± 0.67	164.0 ± 1.41	3.21 ± 0.07	3.75 ± 0.21	0.68 ± 0.11
	6	8.17	311.7 ± 0.52	170.0 ± 2.83	3.92 ± 0.20	4.30 ± 0.14	1.15 ± 0.0
	7	8.32	299.9 ± 1.50	163.0 ± 1.41	2.74 ± 0.07	2.35 ± 0.21	0.70 ± 0.07
overall mean ± 1 SD		8.24 ± 0.059	302.7 ± 5.39	164.1 ± 3.23	3.38 ± 0.67	3.47 ± 0.68	0.96 ± 0.32

Table 11. Comparisons (t-test) between milt plasma and ovarian fluid with respect to osmolality and ionic composition (Phase II).

Parameters	Milt plasma	Ovarian fluid	P
pH	7.83	8.24	<0.01
Osmolality (mOsm/kg)	295.3	302.7	<0.05
Na^+ (mM/L)	131.1	164.1	<0.01
K^+ (mM/L)	30.35	3.38	<0.01
Ca^{2+} (mM/L)	0.94	3.47	<0.01
Mg^{2+} (mM/L)	1.10	0.96	n.s.

Table 12. Correlation coefficients (r) between fertilization success (arcsin transformed %) and osmolality and ion concentrations in milt plasma and ovarian fluid samples (** significant, P<0.01) (Phase II).

Parameter correlated with percentage of fertilization success (arcsin transformed %)	Milt plasma (d.f. = 4)	Ovarian fluid (d.f. = 5)
pH	-0.758	+0.105
Osmolality (mOsm/kg)	+0.280	+0.05
Na^+ (mM/L)	+0.399	-0.121
K^+ (mM/L)	-0.008	+0.448
Ca^{2+} (mM/L)	+0.707	-0.098
Mg^{2+} (mM/L)	-0.979**[a]	+0.659

[a]Correlation removed with removal of male no. 4, considered abnormal.

Table 13. Compilation of data on ion concentrations and osmolality levels for chum salmon held in salt water and fresh water: females--blood plasma and ovarian fluid; males--blood plasma and milt plasma.

	Osmolality (mOsm/kg)	Na^+ (mM/L)	K^+ (mM/L)	Ca^{2+} (mM/L)	Mg^{2+} (mM/L)	Cl^- (mM/L)	Ref.
			FEMALES				
Sea water							
Blood plasma	350±2.3	166.8±1.8	1.0±0.07	3.5±0.31	1.5±0.07	136±1.5	
Ovarian fluid	344±6.8	178±3.3	6.3±0.20	3.3±0.26	0.7±0.09	161±2.4	1
	394±44	176±23	5.3±3.5	2.6±0.7	2.9±1.0	-	2
Fresh water							
Blood plasma	294±2.4	143±1.6	1.2±0.26	1.8±0.10	0.6±0.02	112±1.6	1
Ovarian fluid	311±51	141±24	3.2±0.5	1.5±0.6	1.1±0.3	-	2
	302.7±5.4	164.1±3.2	3.38±0.67	3.47±0.68	0.96±0.32	-	3
	291±2.9	146±4.1	4.0±0.26	1.7±0.17	0.5±0.05	116±2.0	1
			MALES				
Sea water							
Blood plasma	370±2.4	167±2.0	1.6±0.24	3.9±0.11	2.4±0.21	144±5.5	4
Milt plasma	375±4.4	162±3.7	72.1±2.78	1.0±0.14	9.5±0.33	178±6.4	4
Fresh water							
Blood plasma	348±3.6	151±2.0	1.6±0.46	4.6±0.20	1.6±0.13	118±1.51	4
Milt plasma	332±5.1	142±1.7	66.1±4.90	2.2±0.10	1.6±0.10	134±1.33	4
	295.3±12.1	131.1±3.61	30.35±3.2	0.94±0.22	1.10±0.54	-	3

References: 1--Hirano et al. (1978), 2--Stoss and Fagerlund (1982), 3--this paper (poolpen fish), 4--Morisawa et al. (1979).

Appendix Table I. Information obtained from monitoring the normal procedures employed for hatchery collection of gamete milt collection and storage. Source: S—seapen, P—pool.

Date	Sample Number	Source	Temperature (°C) Air	Water	Time milt held in body cavity (min)	Temperature of milt at collection (°C)	Temperature of milt on arrival to hatchery (°C)	Duration of exposure of milt samples to ambient air temperature (min)	Total sto time (m collectio fertilizat (hr)
Oct. 24	1	P	17	11	14	15	13.8	4	4.93
	2	P	17	11	13	14	13.8	5	4.95
	4	P	17	11	17	14	13.8	6	4.82
	5	P	17	11	1	13.8	13.8	20	4.80
	6	P	17	11	0	14.5	13.8	21	4.78
	7	P	17	11	4	13.0	13.8	30	4.80
	8	P	17	11	10	14.0	13.8	5	4.78
	10	P	17	11	4	13.5	13.8	3	4.67
	11	P	17	11	3	13.5	13.8	2	4.80
	12	P	17	11	5	13.3	13.8	5	4.73
	13	P	13	10.5	3	13.0	11.9	14	4.03
	15	P	13	10.5	2	12.8	11.9	8	3.93
	17	P	13	10.5	8	12.8	11.9	2	4.14
	19	P	13	10.5	1	12.5	11.9	4	4.18
	22	P	13	10.5	0	12.5	11.9	5	3.93
	25	P	13	10.5	3	13.0	11.9	2	4.07
	26	P	13	10.5	5	12.7	11.9	0	4.10
	29	P	13	10.5	2	13.0	11.9	1	4.13
Oct. 26	1	P	8.5	10.0	-	10.2	-	4	3.89
	2	P	8.5	10.0	-	10.0	-	1	3.85
	3	P	8.5	10.0	-	10.2	-	14	4.07
	4	P	8.5	10.0	-	10.0	-	1	3.92
	5	P	8.5	10.0	-	10.2	-	0	3.82
	11	P	10.8	11.0	17	12.2	10.6	3	5.77
	15	P	8.5	10.0	-	10.2	-	6	3.93
	16	P	8.5	10.0	-	10.0	-	4	3.71
	17	P	8.5	10.0	-	10.2	-	3	4.08
	18	P	8.5	10.0	-	10.0	-	14	3.95
	19	P	8.5	10.0	-	10.0	-	13	3.90
	20	P	8.5	10.0	-	10.8	-	12	3.88
	21	P	8.5	10.0	-	10.5	-	6	4.13
	22	P	8.5	10.0	-	10.0	-	2	3.98
	23	P	10.8	11.0	2	11.8	10.6	5	5.63
	25	P	10.8	11.0	1	12.0	10.6	7	5.67
	26	P	10.8	11.0	3	12.8	10.6	4	5.78
	28	P	10.8	11.0	14	12.8	10.6	6	5.82
	30	P	10.8	11.0	6	12.0	10.6	13	5.77
	40	P	10.8	11.0	36	11.5	10.6	6	4.02

Appendix Table I. (cont'd)

Date	Sample Number	Source	Temperature (°C) Air	Water	Time milt held in body cavity (min)	Temperature of milt at collection (°C)	Temperature of milt on arrival to hatchery (°C)	Duration of exposure of milt samples to ambient air temperature (min)	Total storage time (milt collection to fertilization (hr)
Oct. 26	41	P	10.8	11.0	43	10.9	10.6	7	3.90
	45	P	10.8	11.0	20	11.8	10.6	7	4.18
	46	P	10.8	11.0	27	11.9	10.6	15	4.17
	47	P	10.8	11.0	16	12.2	10.6	26	4.23
	48	P	10.8	11.0	11	11.5	10.6	15	4.32
Oct. 28	14	P	9.5	9.0	10	10.8	7.7	14	4.28
	15	P	9.5	9.0	22	10.7	-	2	4.08
	16	P	9.5	9.0	8	11.0	8.3	16	4.09
	17	P	9.5	9.0	18	10.3	-	5	4.00
	18	P	9.5	9.0	13	10.8	-	11	4.01
	19	P	9.5	9.0	14	10.8	8.7	8	4.18
Oct. 29	25	P	7.8	9.0	-	10.0	9.4	-	5.53
	26	P	7.8	9.0	-	10.0	9.4	-	5.0
	28	P	7.8	9.0	-	10.0	9.4	-	4.9
	29	P	7.8	9.0	-	10.0	9.4	-	4.62
	30	P	7.8	9.0	-	9.7	9.4	-	4.95
	32	P	7.8	9.0	-	9.5	9.4	-	4.85
	33	P	7.8	8.8	50	10.0	9.4	-	3.17
	35	P	7.8	8.8	50	10.0	9.4	-	3.17
	37	P	7.8	8.8	53	10.0	9.4	-	3.33
	38	P	7.8	8.8	55	10.0	9.4	-	3.30
	39	P	7.8	8.8	21	11.0	9.4	-	3.83
	42	P	7.8	8.8	37	10.8	9.4	-	-
Nov. 3	1	P	10.6	9.9	-	10.0	-	25	-
	2	P	10.6	9.9	-	10.2	-	20	-
	3	P	10.6	9.9	-	10.3	-	17	-
	4	P	9.2	9.9	-	11.2	-	26	-
	5	P	9.2	9.9	-	11.0	-	23	-
	6	P	9.2	9.9	-	11.0	-	21	-
	7	P	9.2	9.9	-	10.7	-	18	-
	8	P	9.2	9.9	-	11.3	-	4	-

Appendix Table I. (cont'd)

Date	Sample Number	Source	Temperature (°C) Air	Water	Time milt held in body cavity (min)	Temperature of milt at collection (°C)	Temperature of milt on arrival to hatchery (°C)	Duration of exposure of milt samples to ambient air temperature (min)	Total storag time (milt collection t fertilizatio (hr)
Nov. 4	10	P	10.0	9.5	-	10.2	-	30	4.38
	11	P	10.0	9.5	-	11.0	-	24	4.23
	14	P	10.0	9.5	-	10.3	-	18	3.98
	15	P	10.0	9.5	-	10.0	-	25	4.10
	16	P	10.0	9.5	-	10.5	-	20	4.27
	18	P	10.0	9.5	-	10.0	-	27	4.13

Appendix Table II. Information obtained from monitoring the normal procedures for hatchery collection of gametes: (II) egg collection and storage. Source: S—Seapen, P—pool.

Date	Bucket number	Source	Temperature (°C) Air	Temperature (°C) Water	Time eggs held in body cavity (min)	Temperature of eggs at collection (°C)	Temperature of eggs on arrival at hatchery (°C)	Weight of eggs in bucket (kg)	Total storage time (egg collection to fertilization (hr)
Oct. 24	1	S	17	13.2	5.0	14.0	-	4.66	5.25
	2	S	17	14.2	4.5	14.0	-	4.24	5.37
	3	S	17	14.2	10.0	16.0	-	4.85	5.25
	4	S	17	14.2	20.0	15.0	-	4.84	4.85
	6	S	17	14.8	5.0	16.0	-	4.26	3.97
	7	P	14	10.5	7.0	11.0	-	4.61	3.48
	8	P	14	10.5	12.5	11.5	-	4.80	3.51
	9	P	14	11.5	29.0	11.5	-	5.88	3.08
Oct. 26	1	S	9.8	13.0	5.0	13.8	11.8	-	4.38
	2	S	9.8	13.0	1.5	13.9	11.8	4.21	4.57
	3	S	10.5	12.0	2.0	13.2	11.8	4.41	4.28
	4	S	10.1	12.0	10.0	13.2	11.8	4.32	3.58
	6	S	10.0	12.8	2.0	13.1	10.5	4.72	8.96
	7	S	11.1	12.9	4.0	13.3	10.5	4.38	8.77
	8	S	10.9	12.9	3.0	13.0	10.5	4.63	6.30
	9	S	11.0	12.8	4.5	13.2	10.5	5.07	6.67
	50	P	10.5	13.0	23.0	12.5	10.5	4.47	7.85
	51	P	10.5	13.0	25.0	12.5	10.5	3.32	7.68
	53	P	9.2	11.2	26.0	11.0	10.5	4.03	3.63
	54	P	9.4	11.0	57.0	11.0	10.5	3.77	4.00
Oct. 28	20	P	8.8	9.7	31.0	9.9	8.8	3.32	3.86
	21	P	7.9	9.5	20.5	9.6	8.8	4.16	3.73
	22	P	7.7	9.8	13.5	9.6	8.8	3.66	3.99
	23	P	8.1	9.5	6.0	9.6	8.8	3.82	2.87
Oct. 29	24	P	6.0	8.8	23.0	8.8	-	3.74	5.20
	25	P	6.0	8.8	24.0	8.8	-	3.23	5.13
	26	P	5.8	8.8	28.0	8.8	-	3.71	5.03
	27	P	6.2	8.7	12.5	8.8	-	3.64	4.73
	28	P	6.8	8.7	14.5	8.8	-	3.35	4.54
	30	P	7.5	8.8	17.5	9.3	9.1	4.40	3.50
	31	P	7.6	8.8	18.0	9.1	9.1	4.66	3.83
	32	P	8.0	9.2	10.0	9.2	9.1	3.89	3.45
	34	P	9.1	9.0	4.0	9.5	9.1	3.29	3.18
	35	P	9.1	9.0	2.0	9.3	9.1	4.63	3.17

Appendix Table II. (cont'd)

Date	Bucket number	Source	Temperature (°C) Air	Water	Time eggs held in body cavity (min)	Temperature of eggs at collection (°C)	Temperature of eggs on arrival at hatchery (°C)	Weight of eggs in bucket (kg)	Total storage time (egg collection to fertilization (hr)
Nov. 3	51	P	9.8	10.0	47.0	9.9	-	-	6.24
	52	P	10.6	10.1	50.0	9.9	-	-	6.57
	53	P	10.0	9.9	12.0	11.1	-	-	4.86
	55	P	9.2	9.9	43.0	11.6	-	-	2.87
	56	P	9.2	9.2	6.0	9.9	-	-	3.15
Nov. 4	60	P	8.2	9.6	1.0	9.1	-	4.20	6.63
	61	P	8.2	9.6	33.0	9.4	-	4.21	6.63
	62	P	10.0	9.5	18.0	9.8	-	3.40	5.48
	63	P	10.0	9.5	16.0	9.7	-	2.81	5.42
	64	P	10.9	9.8	10.0	9.8	-	4.15	4.4
	65	P	10.9	9.8	15.5	9.8	-	3.19	4.33

Appendix Table III. Information obtained from monitoring the normal procedures for hatchery collection of gametes: (III) fertilization of gametes.

Date	Eggs bucket number	Milt sample numbers	Temperature of gametes at fertilization (°C)		Presence of turgid eggs before fertilization (%)	Contact time of gametes, fertilization to activation: (min)	Interval between activation and transfer to incubator (min)	Fertilization success (%)	Stage of development preserved for evaluation of fertilization success
			Eggs	Milt					
Oct. 24	1	1,2,4	14.3	10.0	13.8	3.5	1.5	0.0	2-cell
	2	5,6	12.7	9.6	1.3	4.0	0.5	55.2	2-cell
	3	7,8,10	13.2	9.5	0.0	2.0	0.5	40.0	2-cell
	4	10,11,12	12.8	9.4	0.0	3.0	0.5	66.7	2-cell
	6	10,11,12	12.8	9.4	11.6	2.0	4.0	44.8	2-cell
	7	13,15,22	11.5	10.1	1.0	4.5	0.5	83.3	2-cell
	8	25,26,29	11.4	9.3	0.0	3.0	0.5	86.6	2-cell
	9	17,19,22	10.5	9.2	0.9	4.5	0.5	44.4	2-cell
Oct. 26	1	1,2,15, 16	11.0	8.5	0.0	3.5	0.5	95.2	Caudal bud free
	2	3,18,19, 20	10.9	8.5	3.5	3.0	0.5	71.4	Caudal bud free
	3	3,18,19, 20	10.6	8.5	0.0	2.0	1.0	93.5	Caudal bud free
	4	4,5,17, 21,22	10.5	8.5	3.6	2.5	0.5	71.4	Caudal bud free
	6	N/A	9.2	8.9	0.0	3.0	0.5	91.7	Caudal bud free
	7	N/A	9.4	8.9	0.0	2.0	1.0	97.7	Caudal bud free
	8	40,41,46	9.4	8.7	6.4	3.0	0.5	47.9	Caudal bud free
	9	45,47,48	9.9	7.8	3.2	4.0	0.5	84.4	Caudal bud free
	50	11,26,28	9.7	8.5	4.6	1.5	0.5	72.2	Caudal bud free
	51	23,25,30	9.7	8.5	1.2	1.5	0.5	98.0	Caudal bud free
	53	23,25,30	9.1	8.5	1.1	0.5	0.5	85.3	Caudal bud free
	54	45,47,48	10.3	7.8	0.0	2.0	0.5	95.5	Caudal bud free

Appendix Table III. (cont'd)

Date	Eggs bucket number	Milt sample numbers	Temperature of gametes at fertilization (°C)		Presence of turgid eggs before fertilization (%)	Contact time of gametes, fertilization to activation: (min)	Interval between activation and transfer to incubator (min)	Fertilization success (%)	Stage of development preserved for evaluation of fertilization success
			Eggs	Milt					
Oct. 28	20	16,17,18	8.6	8.6	0.0	2.5	1.0	100.0	epiboly complete
	21	16,17,18	8.6	8.6	0.0	2.5	0.5	98.0	epiboly complete
	22	14,15,19	8.5	8.2	0.0	1.5	0.5	97.6	epiboly complete
	23	14,15,19	9.2	8.2	1.3	1.5	0.5	91.3	epiboly complete
Oct. 29	24	25,28,29 30	8.2	8.6	0.0	2.0	1.0	97.3	3/4 epiboly
	25	26,32	8.2	8.5	0.0	1.0	0.5	100.0	3/4 epiboly
	26	N/A	8.2	8.7	0.0	1.0	0.5	94.9	3/4 epiboly
	27	N/A	8.1	8.6	2.9	1.5	0.5	96.2	3/4 epiboly
	28	N/A	8.1	8.6	0.0	0.5	0.5	98.9	3/4 epiboly
	30	33,35,42	8.8	9.4	0.0	1.5	0.5	100.0	3/4 epiboly
	31	37,38,39	8.9	9.3	1.1	1.0	0.5	97.8	3/4 epiboly
	32	33,35,42	9.1	9.4	0.0	2.0	1.0	100.0	3/4 epiboly
	34	37,38,39	9.1	8.5	0.0	1.5	0.5	98.2	3/4 epiboly
	35	37,38,39	9.3	8.5	0.0	2.0	0.5	98.0	3/4 epiboly
Nov. 3	51	N/A	9.8	N/A	0.0	2.0	0.5	97.0	epiboly complete
	52	N/A	9.5	N/A	0.0	1.0	0.5	97.4	epiboly complete
	53	N/A	9.8	N/A	0.0	0.5	1.0	99.1	epiboly complete
	55	N/A	9.8	N/A	0.9	1.0	0.5	94.6	epiboly complete
	56	N/A	9.8	N/A	0.8	1.5	0.5	97.5	epiboly complete
Nov. 4	60	3,10,11, 16	N/A	N/A	0.0	1.0	0.5	96.4	3/4 epiboly
	61	8,14,15, 18	N/A	N/A	0.0	1.5	0.5	97.2	3/4 epiboly
	62	8,14,15, 18	N/A	N/A	1.1	0.5	0.5	94.6	3/4 epiboly
	63	3,10,11, 16	N/A	N/A	0.0	1.0	0.5	95.2	3/4 epiboly
	64	8,14,15, 18	N/A	N/A	0.0	1.0	0.5	100.0	3/4 epiboly
	65	8,14,15, 18	N/A	N/A	0.0	1.0	0.5	98.6	3/4 epiboly

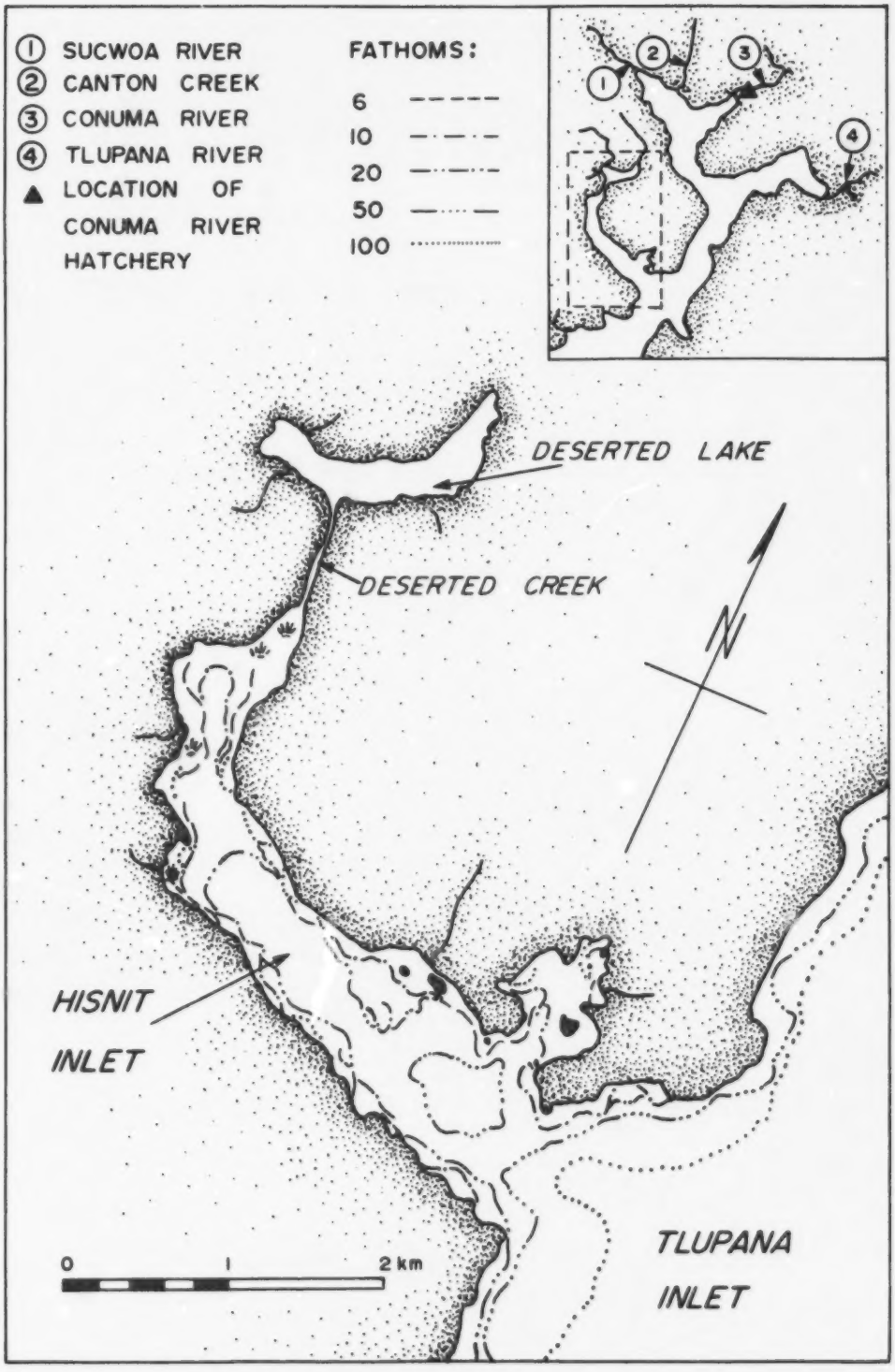

Fig. 1. Location of Deserted Creek, Hisnit Inlet, B.C.

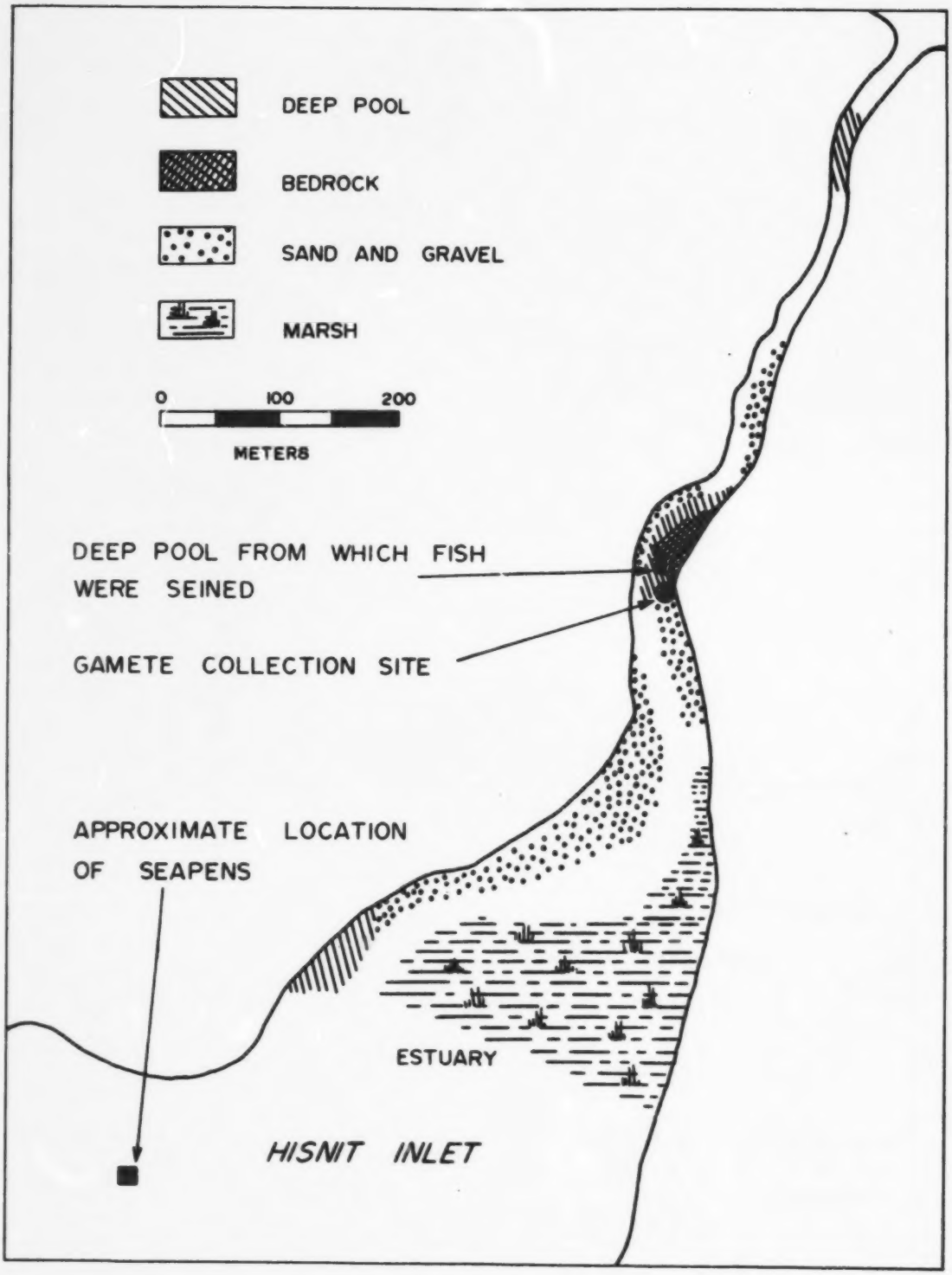

Fig. 2. Deserted Creek, indicating location of the seapen and pool sites.

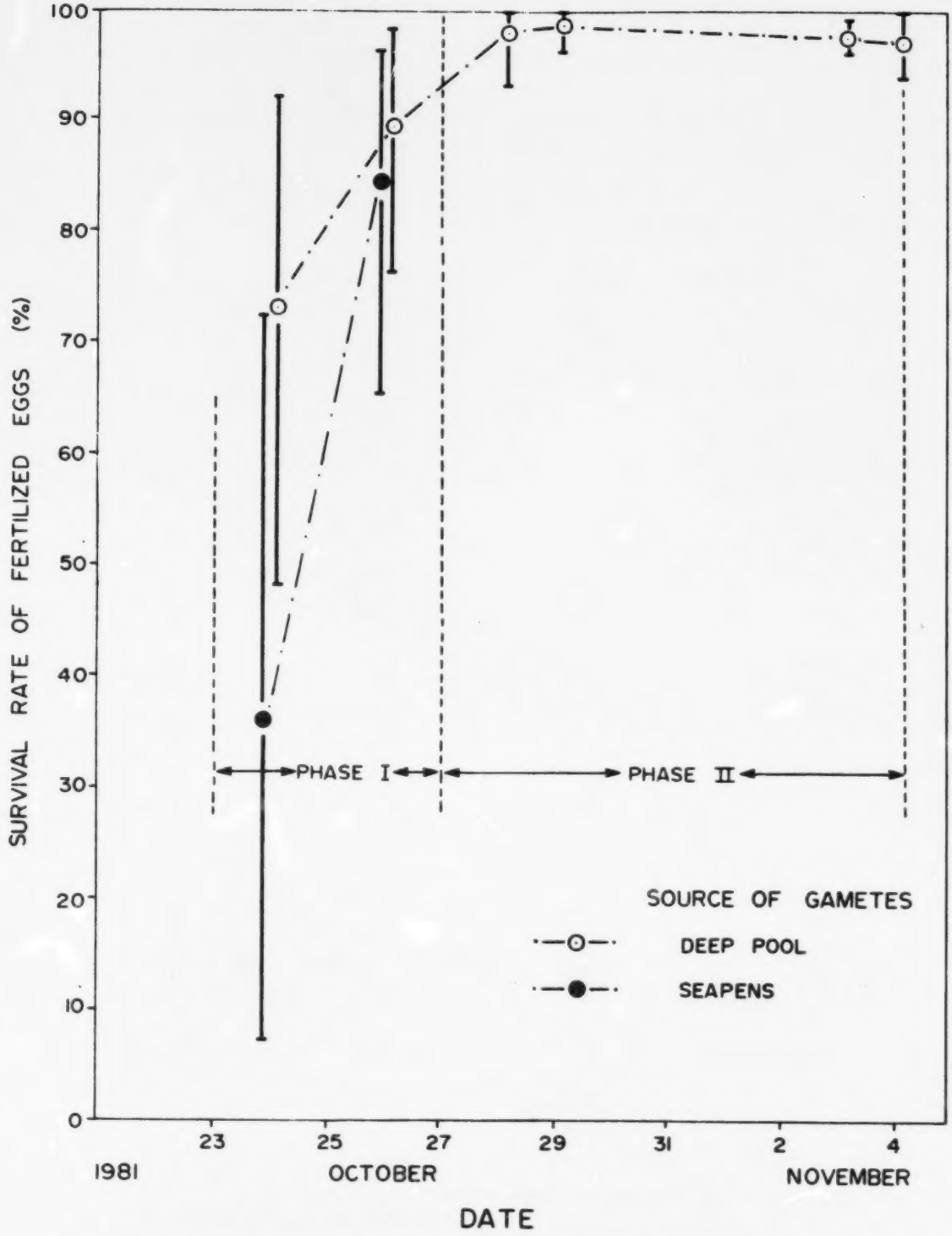

Fig. 3. Changes in gamete viability during the spawning period. Survival of eggs after fertilization: mean ± SD (%). Seapen and pool sites.

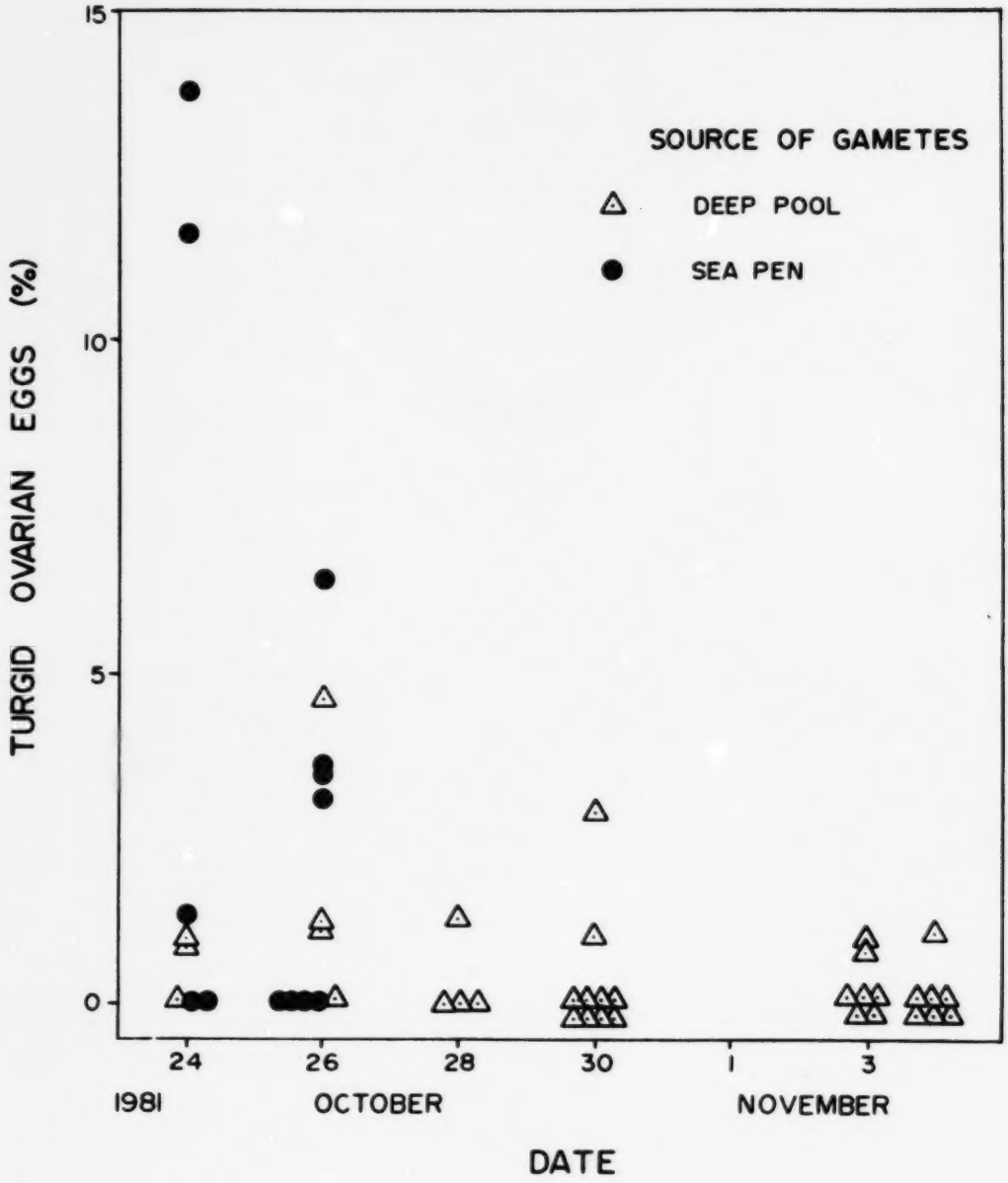

Fig. 4. Occurrence of turgid eggs in samples from the two holding sites for adults.

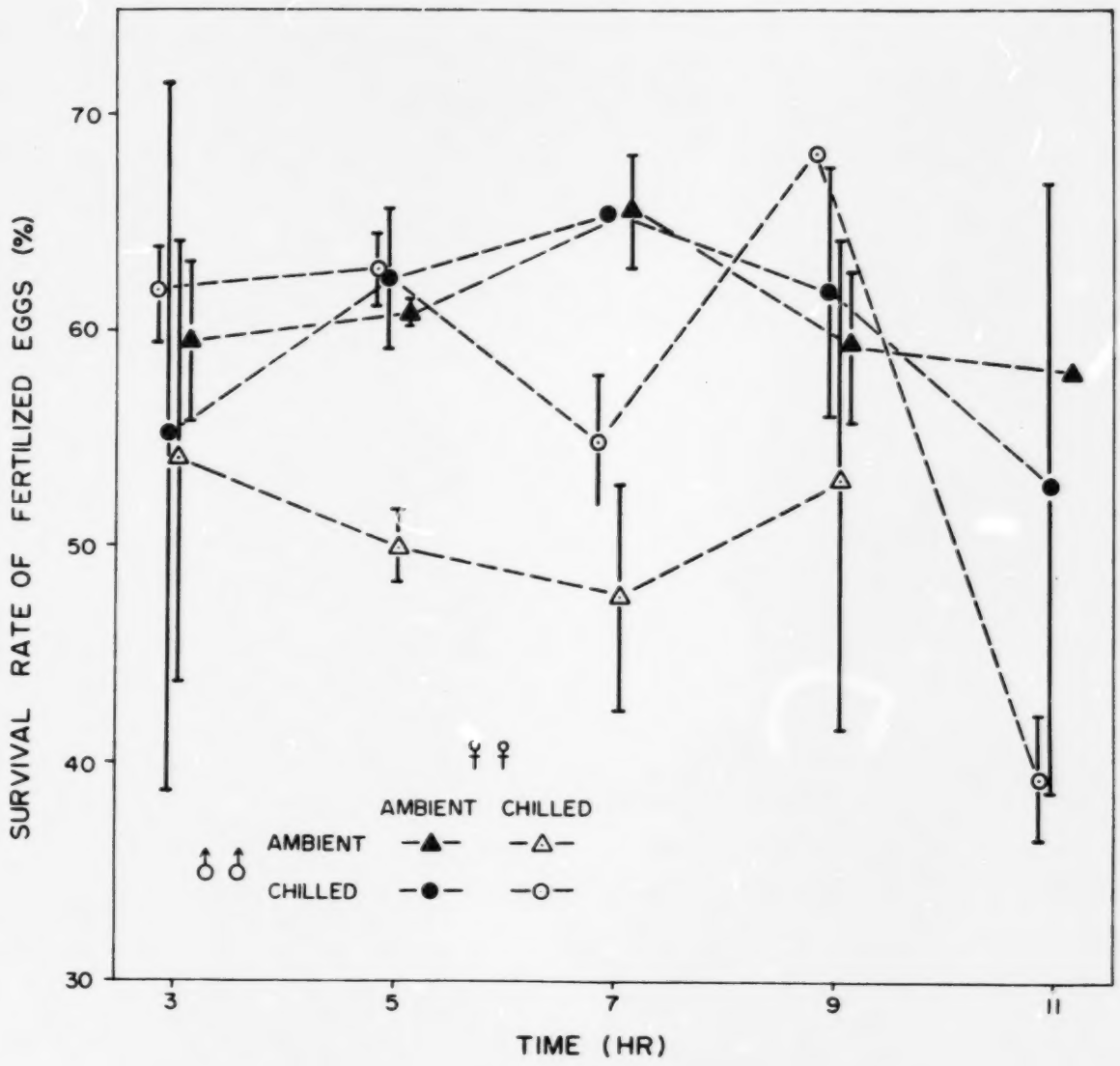

Fig. 5. Fertilization success of stored gametes in relation to storage temperature (ambient, A; chilled, C). Gametes collected from the seapen site (means ± 90% CI).

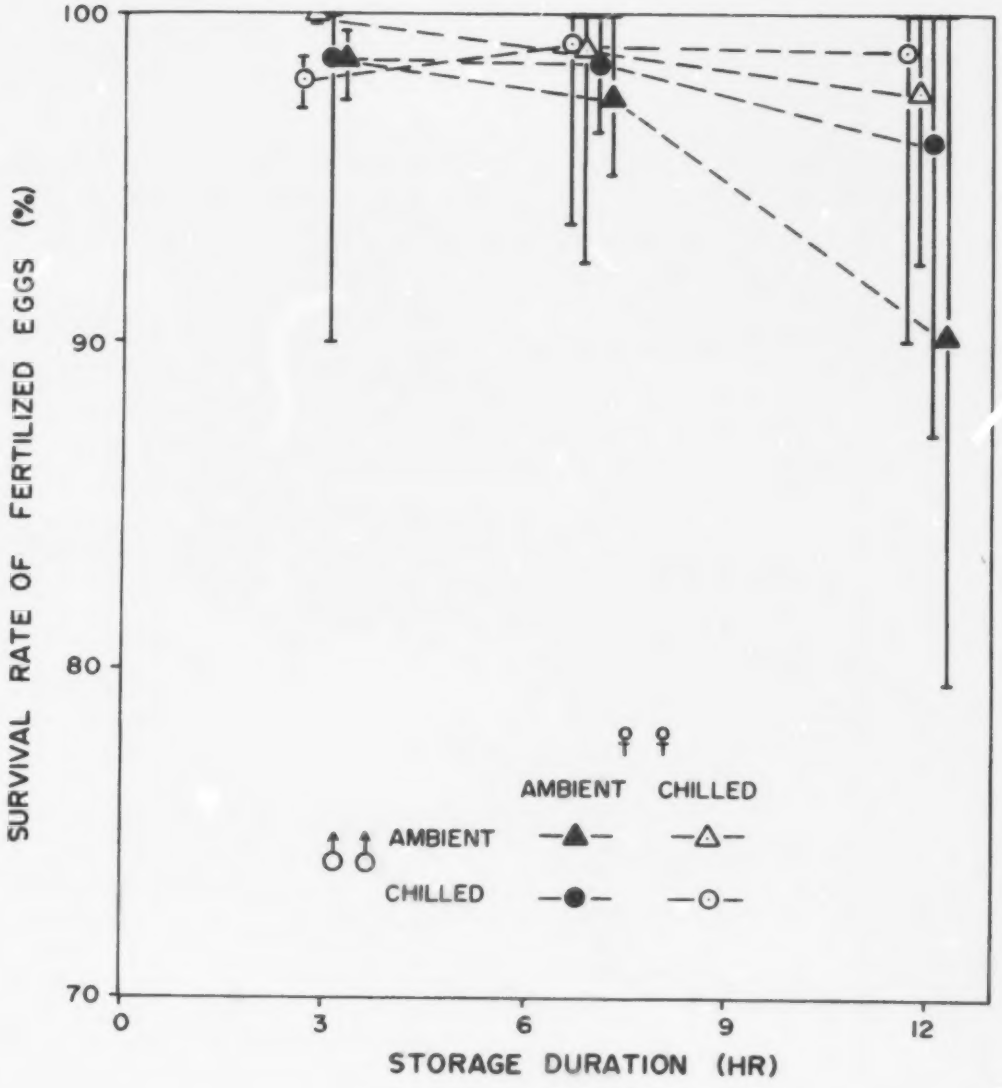

Fig. 6. Fertilization success of stored gametes in relation to storage temperature (ambient, A; chilled, C). Gametes collected from the pool site (means ± 90% CI).

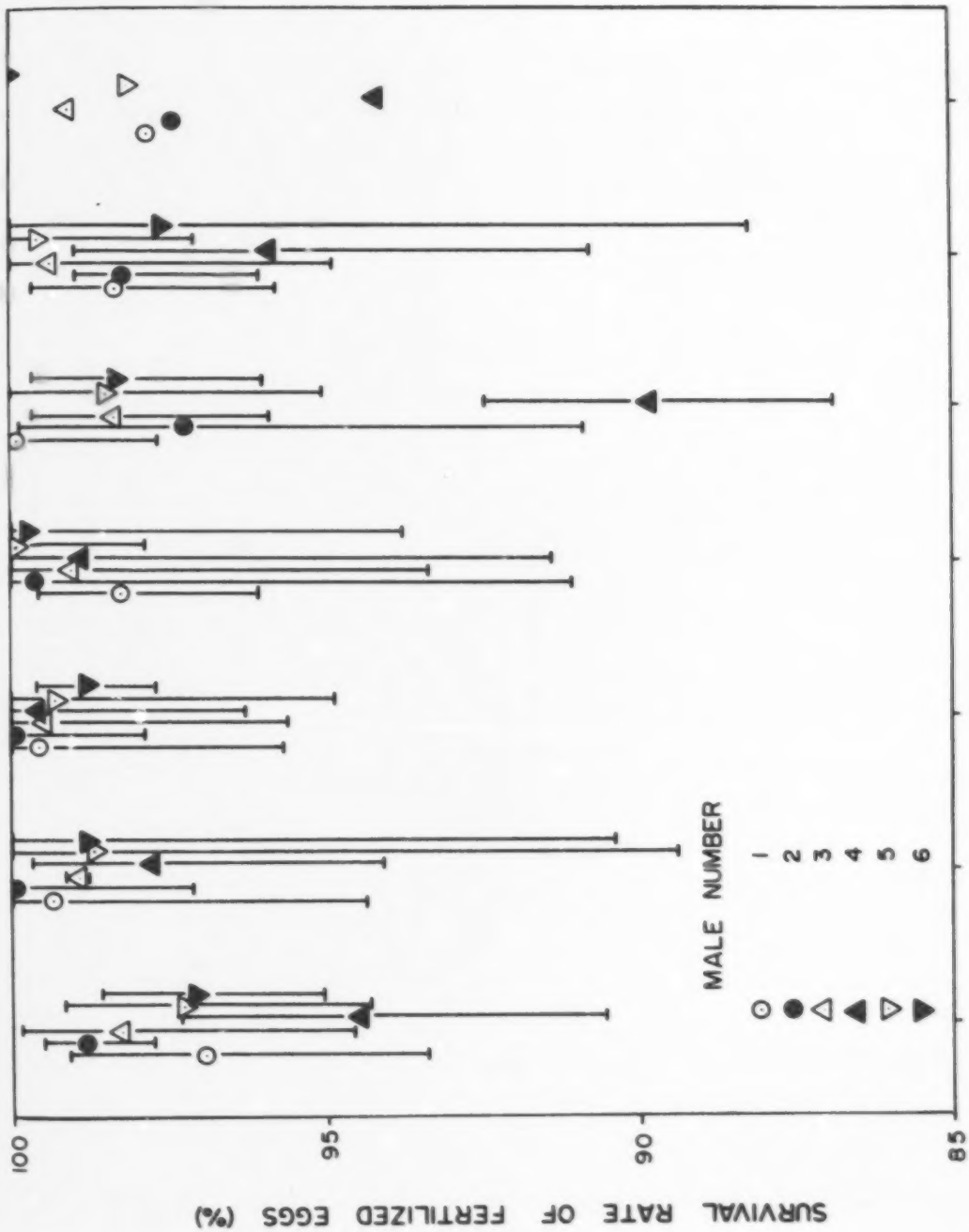

Fig. 7. Fertilization success in 42 crosses of seven females and six males in which ion composition of ovarian fluid and milt plasma was determined. Means ± 90% CI.

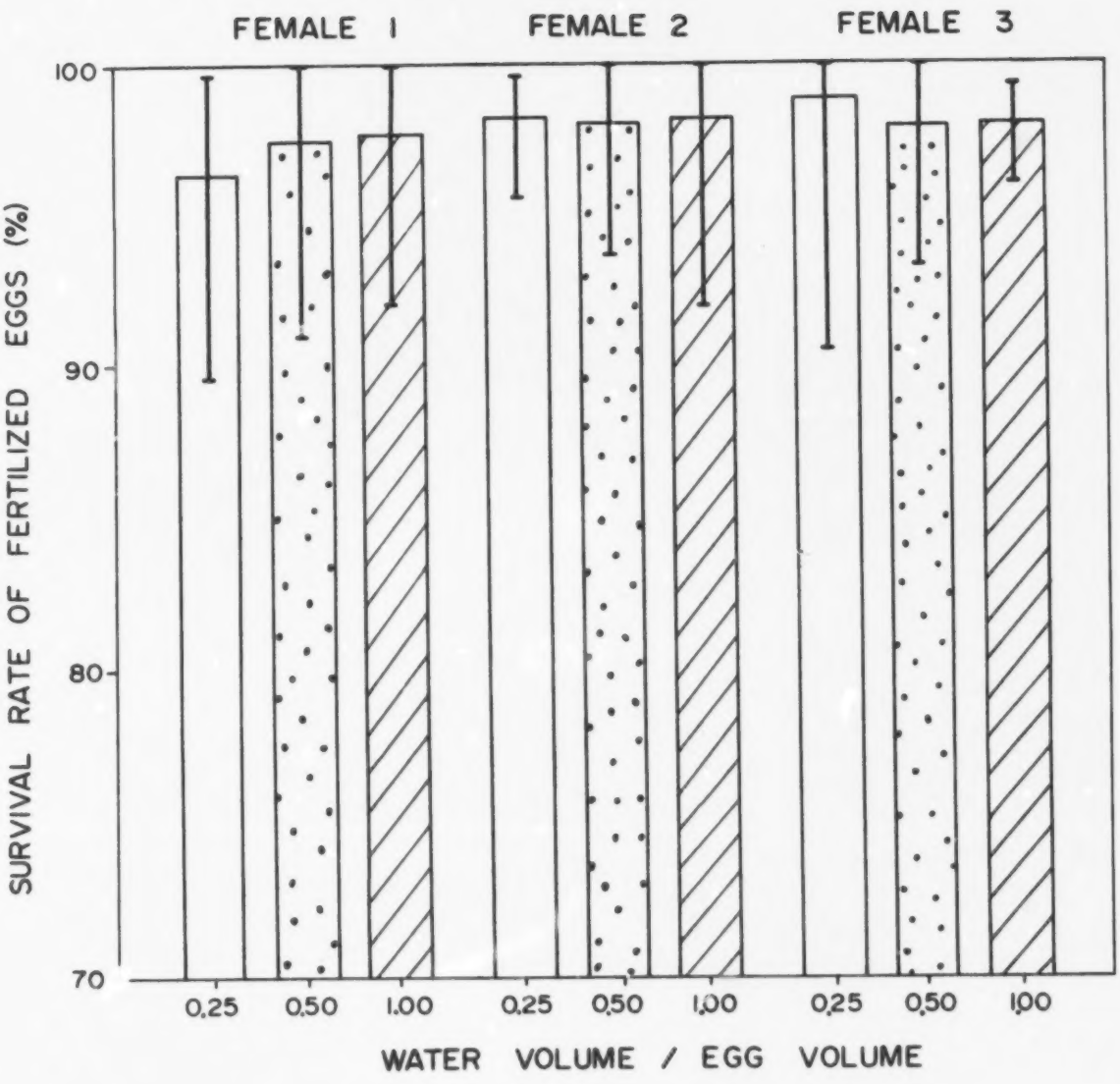

Fig. 8. Fertilization success of eggs activated at three different water volume/egg volume ratios.